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SOIL SCIENCE

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JULIUS STOKLASA

UNIV. OF MICH.

Julius Stoklasa[1]

1860-1936

One of the greatest agricultural scientists and one of the most interesting personalities of our day, Dr. Julius Stoklasa, died in Prague, April 4.

Stoklasa began his professional studies in Tetschen in 1873. After two years he entered the Agricultural Institute in Vienna. Later he studied in Leipzig, under Oswald and Pfeffer, and in the Pasteur Institute in Paris, where he began his work in soil biology. It is at these institutions that the young student learned, in the words of Professor Bornemann-Eisenwach, "to understand and comprehend the longing for life, and above all the life of plants."

Stoklasa's research activities extended over a wide field. His first publication, the thesis for his doctorate at Leipzig, "Über die physiologischen Eigenschaften der wasserlöslichen Verbindung der Phosphorsäure in den Superphosphaten," pointed to the future trend of his life work. As a foundation for his work in plant physiology, Stoklasa undertook a study of soil processes. He became particularly interested in the rôle in plant nutrition of certain specific elements; namely, arsenic, aluminum, and iodine. He also investigated various changes which take place in the growing plant, and soon became a pioneer in the field of biochemistry, especially in the study of enzymes. In his studies on the transformation of phosphorus, his attention was called to the importance of bacteria, while his biochemical investigation of the soil led him directly to the study of soil bacteriology, whereby he contributed to making this an independent science. Thus he penetrated further and further into the nature of plant life in his search for the source from which organisms draw their life energy. In time, these studies led to the discovery of the influence of radioactivity on the growth and movement of matter in organisms.

Because of their great number—more than 400—it is impossible here to enumerate the scientific contributions of Stoklasa, which cover an enormous field of research. The commemoration volume published by P. Parey in Berlin in 1928 in honor of Stoklasa's seventieth birthday, gives a comprehensive account of his work. To be sure, this account is now incomplete, since Stoklasa continued to spend the last ten years of his life in diligent research. Among his extensive publications, mention should be made of "Der Biochemische Kreislauf des Phosphat-ions im Boden," "Biophysikalische und biochemische Durchforschung des Bodens," and his work on the smoke damage problem and on the cycle of aluminum in nature. His last investigations dealt with the biology of radium, of which only the first volume has so far appeared.

[1] Prepared by Dr. E. G. Doerell, formerly one of Professor Stoklasa's assistants.

In many instances, Stoklasa developed new methods and propounded theories to answer both general and specific questions. Whether or not one agreed personally with these theories, it must be admitted that they stimulated further scientific investigation. As the alchemists are considered the founders and prophets of modern chemistry, in the same light Stoklasa may be regarded as one of the last great alchemists. The near or distant future will probably verify many of his hypotheses.

In spite of his specialized researches, Stoklasa was anything but one-sided. He was the typical prewar *Grand Seigneur* in appearance and character, a man of the world who embraced the universe in his studies. His life work was devoted to an investigation of the relations between life processes and living systems. It is not by chance, therefore, that in his works he always returned to the soil as the source of life.

Stoklasa was a prominent organizer. He is numbered among the founders of such institutions as the Czechoslovakian Academy of Agriculture and the Technical Museum. He also served as president of the Third Commission of the International Society of Soil Science. He was a brilliant orator who, by his eloquence, captivated not only professional scientists but also practical farmers.

The death of Stoklasa brings to a close a life rich in results and in numerous honors. The spirit of Stoklasa, whose life work was to fathom the "Spirit of the Soil," will continue, through his work, to influence the development of soil science.

SEASONAL CHANGES OF THE MOISTURE CONTENT OF SOLAGA SOIL OF PALESTINE AND THEIR INFLUENCE ON VEGETATION

FELIX MENCHIKOVSKY

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Received for publication June 2, 1936

The deficiency of soil moisture is the chief factor which determines the presence and development of plant life under the soil conditions of Palestine. With the exception of that on irrigated land the vegetation corresponds to the season as well as to the total rainfall during the year and the amount of moisture preserved in the soil during the dry season.

In the Beer Sheba district, for instance, which is distinguished by low rainfall and high permeability of loess soil, the cultivation methods of the native population aim toward the accumulation of water in the depths of the soil and its preservation from evaporation.

These methods empirically found and others newly introduced into Palestinian practice may be accepted only after detailed study of the water balance in the soils. With this in view, the moisture content and the amount of soluble salts were recorded under field conditions in the different depths of loamy Solaga soil for two years.

THE PROPERTIES OF SOLAGA SOIL

The Solaga soil, the water retaining conditions of which were studied, belongs to the common type of heavy soils of the coastal plain. The study of the soil profile indicates the presence of two layers as follows:

The upper layer, which reaches a depth of 40 cm., is distinguished by a good structure. This layer is composed of aggregates of particles and is perforated with channels, formed by plant roots. Being continually cultivated, it shows better physical properties than the subsoil layers of the soil profile.

The lower layer, below a depth of 40 cm., appears as a structureless heavy soil which absorbs much water and swells extensively.

The study of the nature of exchangeable bases in this soil has shown that the upper layer contains more exchangeable Ca and less exchangeable Na. than the lower layer. The average annual rainfall in the belt of this soil is about 500 mm.

ANNUAL CHANGES IN WATER CONTENT OF DIFFERENT LAYERS OF SOLAGA SOIL

Samples of soil from different layers of the most typical locality were collected from a certain plot which had not been cultivated. In order to get a

distribution of the soil moisture, unaffected by vegetation, we invariably destroyed the wild plants springing up during the rainy season. The collected samples were examined, and the moisture content as well as the amount of soluble salts in the soil was recorded.

The graphical interpretation of these records, in figure 1, shows the character of the distribution of the soil moisture in different depths during different seasons of the year. The months when the soil samples were collected are represented on the abscissa, and the moisture of soil examined, expressed in percentage, on the ordinate. The four curves thus obtained correspond to the four layers of the soil *within 25 cm. of one another* and show the annual *courses* of the moisture content in different layers of Solaga soil.

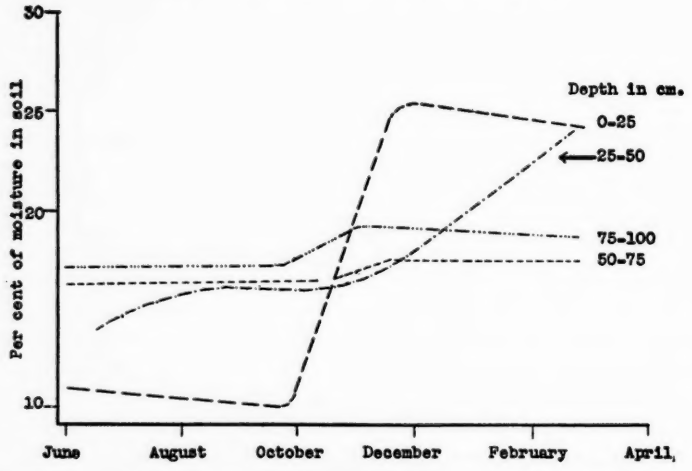

FIG. 1. ANNUAL COURSES OF THE MOISTURE CONTENT OF DIFFERENT LAYERS OF SOLAGA SOIL

The curve for the 0–25 cm. depth indicates that in the month of June there is about 11 per cent of moisture in the soil. This figure gradually decreases until by October only 9.5 per cent remains, representing the lowest water content found in this type of soil. June–October is the dry season and the hottest time of the year in Palestine. The value of night dew in the formation of soil moisture has not yet been studied here. In any case, the dew does not increase the moisture content of the surface layer, and the heat of the following day removes the last traces of non-hygroscopic water of the soil. In November the rainy season begins, and the curve of moisture shows a sharp rise at this time, continually increasing until December, when the maximum is reached. It subsequently slowly decreases until the end of the dry season. The course of the curve illustrates well the yearly changes in water content of the surface soil in Palestine.

The curve for the second layer, i.e., the 25–50 cm. depth, is different from the one just described. The lowest figure, which is 13 per cent, found in the month of June, shows that the minimum of moisture in the second layer is greater than the minimum in the first (0–25 cm.) for the same season. The course of this curve shows a constant increase until the month of March, when the moisture content of the soil reaches its maximum of 23 per cent. In contrast to the 0–25 cm. layer, the moisture content of which continually decreases during the June–October period, the moisture of the 25–50 cm. layer continually increases during the same interval. This increase of water content in the 25–50 cm. depth can be explained by the process of distillation. Soil moisture from the surface layer, continually heated, becomes vapor, which in turn condenses in the lower layer. Lebedev (2) found that in cases where the water content of the soil reaches the maximum hygroscopicity, the relative humidity of the soil air reaches 100 per cent. According to his data, the soil of southern Russia even at a depth of 5–10 cm., is saturated with water vapor. As a rule, the temperature of the soil decreases with the depth, and the pressure of the water vapor declines with it. Possessing higher pressure, the water vapor of the upper layer moves to the lower layer, where it condenses. The second part of the curve belongs to the rainy season. It shows that the rains increase the moisture content of the soil to a lesser degree than in the previous layer. This difference can be explained by the physicochemical properties of the soil, which cause it to swell greatly and thus prevent the passage of the water to the subsoil.

The curve for the third layer, i.e., 50–75 cm., has an entirely different character. The moisture in this depth remains within the limits of 15.8 to 17.0 per cent throughout the year. The influence of the rainy season is manifested here only by a slight increase of moisture in the soil (about 1.0 per cent).

The curve for the 75–100 cm. layer generally resembles the course of the 50–75 cm. curve, the figures, however, being relatively higher.

SEASONAL CHANGES IN WATER-SOLUBLE SALTS IN THE SOIL

The general content of water-soluble salts is a distinctive factor in determining soil types as well as conditions of plant growth. The seasonal changes in temperature and in amount of rainfall influence the amount and concentration of soluble salts in the soil at different depths.

The quantities of soluble salts in the plot were determined, and the concentration of the salts in the liquid phase of the soil was calculated for the dry and wet seasons.

The first curve (0–25 cm.) in figure 2 shows that for the June–October period the concentration of soluble salts is similar to that of soils in arid regions whereas during the November–March season it is similar to that of soils in humid regions. The same properties are observed with the three other curves, but less distinctly. The curves showing changes in concentrations of salts in the 50–75 and 75–100 cm. depths during the year were compared with the

corresponding, approximately constant, moisture distribution curves, and thus the *rhythmic up and down movements of the soluble salts in the soil occurring in the course of the year were demonstrated.*

MOLECULAR FORCES OF SOIL AND WATER RETENTION

The fact that the amount of moisture at a shallow depth (50–75 cm.) remains constant during the very dry and hot season of the year when the soil is strongly insolated can be explained by the existence of strong molecular forces which keep the water on the surface of the soil particles. The character of water absorptions in the soil can be seen in table 1, in which moisture of air-dry soil, hygroscopicity according to Mitscherlich, and moisture in the soil

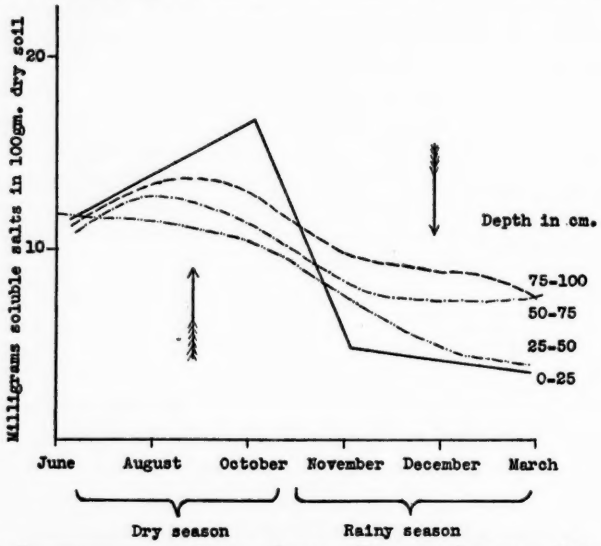

FIG. 2. CONCENTRATIONS OF SOLUBLE SALTS IN DIFFERENT LAYERS OF SOLAGA SOIL
Arrows show the up and down movement of the soluble salts

equivalent to the coefficient of Mitscherlich, as well as fluctuations of soil moisture during the year, were recorded. The proximity of the figures for hygroscopicity according to Mitscherlich to those for the moisture content in the 50–75 cm. layer is especially evident for the dry season. These peculiar properties of the 50–75 cm. soil layer can be explained by the low temperature of the soil, the higher concentration of soluble salts, and the greater proportion of exchangeable Na in the absorbing complex of this layer. These three factors work in unison for the lowering of the water vapor pressure and the preservation of water in the soil. The property of heavy hydration of the soil, the absorbing complex of which is saturated with monovalent bases, has been ascertained by several workers (7). The second and third factors are conse-

quences of the semiarid climatic conditions which are predominant in these soils. We thus arrive at the conclusion that *the greater part of this water is retained by surface forces and is therefore hardly available to the common plants.* According to the classification of Lebedev, this water is partly "hygroscopic" and partly "membrane" water.

DISTRIBUTION OF MOISTURE IN THE SOIL AND SEASONAL CHANGES OF VEGETATION

The wild vegetation of Palestine is distinguished by the change of plant associations in accordance with different climatic and soil conditions during the various seasons of the year. These changes are brought about in the following way (fig. 3):

Most of the tuberous and bulbous plants develop, flourish, and bear fruit from the beginning of October until February.

The annual plants appear in November or December and grow until the end of May, gradually associating themselves with the previous flora of tuberous and bulbous plants. From January to May the annual plants predominate.

TABLE 1

Moisture content of Solaga soil

DEPTH OF SOIL	MOISTURE IN AIR-DRY SOIL	HYGROSCOPICITY ACCORDING TO MITSCHERLICH	MOISTURE IN SOIL EQUIVALENT TO COEFFICIENT OF MITSCHERLICH	FLUCTUATIONS IN MOISTURE CONTENT OF SOIL DURING THE YEAR
cm.	*per cent*		*per cent*	*per cent*
0–25	9.55	16.29	14.0	9.25–25.1
25–50	9.24	15.56	13.5	13.0 –23.2
50–75	9.25	16.02	13.9	15.7 –17.0
75–100	9.74	16.90	14.5	16.3 –19.1

The perennial plants. The beginning of the season of perennial plants coincides with the period of vegetation of the annual plants and continues until the end of September.

In October this vegetation cycle begins again.

The plant associations, mentioned are composed of species adapted to the different soil climatic conditions. Their presence and changes are dependent chiefly on the distribution of soil moisture during the year.

The appearance of the tuberous and bulbous plant formations is possible only in the *humid soil conditions which, as has been shown, exist only during the October–March interval in the 0-25 cm. depth.* The percolation of rain water to the 50 cm. depth during the following months favors the development of the vegetation of annuals, the roots of which reach that depth. The dry season of the June–October period hinders the physiological activity of the bulbous and tuberous plants and prevents the existence of the annual plants, the roots of which take their water from the 0–25 cm. and 25–50 cm. depths. Since our observations proved that during this same season the moisture of the 50–100

cm. depth remains constant throughout the year, the existence of the perennial plants and of those annuals the roots of which reach that depth is possible.

Two sets of field crops, each containing different species of plants, are known in the agricultural practice of Palestine. They are the *summer* and *winter crops*, the growth of which correlates with the aforementioned changes in soil moisture conditions and their corresponding wild vegetation.

The character of the moisture retained by Solaga soil raised the question whether this moisture would, as a rule, be available to the common plant.

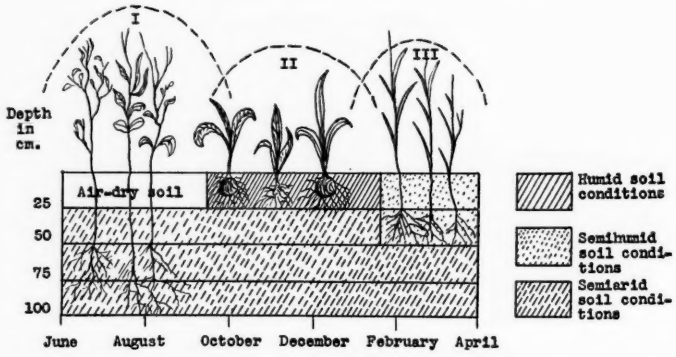

FIG. 3. SEASONAL CHANGES OF THE WILD VEGETATION OF PALESTINE

I—Season of predominant perennial and annual plants with roots longer than 50 cm.
II—Season of predominant bulbous plants.
III—Season of predominant annual plants.

TABLE 2

"Wilting points" of different layers of Solaga soil

DEPTH OF SOIL	WATER ABSORBED BY 100 GM. OF SOIL IN ATMOSPHERE SATURATED WITH VAPOR	WILTING POINT CALCULATED ACCORDING TO FORMULA OF BRIGGS	WATER IN SOIL EQUIVALENT TO WILTING POINT OF BRIGGS	WATER IN SOIL EQUIVALENT TO WILTING POINT ACCORDING TO BOGDANOV
cm.	*gm.*		*per cent*	*per cent*
0–25	18.4	26.6	21.0	19.1
25–50	18.5	26.7	21.1	18.5
50–75	18.3	26.5	20.9	18.5
75–100	17.5	25.3	20.2	19.5

Table 2, which shows the wilting points for the different layers of Solaga soil, throws more light upon the subject. These figures are obtained by the calculation of the amount of moisture absorbed by air-dry soil in the atmosphere saturated with water vapors according to the formula of Briggs (1), and of the doubled hygroscopicity of air-dry soil according to Bogdanov. Only in the 0–25 cm. layer during the October-March period and in the 25–50 cm. layer at the end of the wet season, when the soil reaches its maximum water content, does the amount of moisture in the soil exceed or even reach the wilting point.

In the lower layers of the soil, i.e., 50–100 cm., in depth, the maximum moisture content is much lower than the wilting point and approaches the coefficient of Mitscherlich's hygroscopicity. Since not only hygroscopic water, but also a "distinct part of the capillary water" (3), is generally unavailable to the plants, this water scarcity phenomenon renders the problem of the plant water supply in the heavy soils of Palestine different from the same problems in humid climates.

XEROPHYTIC CHARACTER OF PALESTINE'S VEGETATION

The drought resistance of the plants has been interpreted by Fitting and Maximov as a physicochemical property (5): "The high concentration of cell sap allows the plants to grow in the dry atmosphere and under conditions of insufficient soil moisture. . . . Only through the high osmotic pressure can the xerophytic plant in the wilting state develop an important water absorbing capacity. At the same time the increased concentration of the sap causes dehydration of the protoplasm and membranes, thereby decreasing the loss of water by the plant."

In Palestine the October-March season is the germination period of Gramineae. As already stated, there is a high moisture content, which is propitious

TABLE 3

Ash content of straw

VARIETY OF PLANT	EUROPE	UNITED STATES	PALESTINE
	per cent	*per cent*	*per cent*
Wheat	4.7	4.2	10.6
Barley	5.0	5.7	9.7
Maize	...	3.4	13.6

to root development, in the upper layer of soil (25 cm.) during the rainy season. Later, however, when the roots reach a depth of 50 cm., they enter into layers having a water content little above or even lower than the wilting point. It was natural, therefore, to find in the composition of cell sap some aid to plant growth. The ash content of the plants may, in such a case, give some idea of the concentration of cell sap.

The composition of straw ash in Palestine and in other lands is presented in table 3. The ash content of the straw of wheat and barley is high in comparison with that of European and American crops. This high salt content may play an important part in the establishment of a high osmotic pressure of cell sap, which in turn renders the problem of obtaining moisture from the soil less difficult and enhances the loss of water in the exceedingly dry air of Palestine.

Here, in the unfavorable air and soil conditions, the xerophytic character of the local corn with an ash content which reaches 13.6 per cent is more pronounced than that of any other grain. In Palestine the maize develops during the months of April-August, when the water content of the surface layer of soil

reaches 10.5–9.5 per cent, a state of air dryness. For this period the moisture, as shown in table 1, was preserved only at a depth of 50–100 cm. The necessary moisture for the corn plant, therefore, can be obtained in the following ways: by sending the roots to a depth of 100 cm. or more and through the high osmotic pressure of the cell sap, which permits the absorption of soil moisture, which is lower than the wilting point for plants of humid climates.

SUMMARY

The changes in the dry and wet periods of the year in Palestine influence the moisture content of only the superficial layer of Solaga soil (0–50 cm.).

The greatest changes in moisture content were observed in the 0–25 cm. layer, from a minimum in October (air-dry soil) to a maximum in December (about 24 per cent).

The moisture content of the 50–100 cm. depth remains about constant during the year, with a slight increase during the wet season (about 1 per cent).

The changes of plant associations are closely connected with the soil moisture distribution during the year at different depths. *The bulbous and tuberous plants* vegetate during the short period of high moisture content of the 25 cm. depth during the months of October to March; *the annual plants* the roots of which penetrate not more than to a 50 cm. depth grow from November until May; *the perennial plants* obtain their water mostly from a 50–100 cm. layer depth, where the moisture remains about constant during the year.

With few exceptions (the temperate humid conditions during the wet season at a 25 cm. depth) the normal amount of moisture in Solaga soil is lower than the moisture content equivalent to the wilting point of plants of humid climate. Important elements of the Palestine flora as well as local varieties of agricultural plants possess a more or less pronounced xerophytic character which helps them to exist and develop in the insufficient soil moisture conditions.

The high ash content of some local grains proves their xerophytic character. The high osmotic pressure of their cell sap permits the absorption of the water imbedded in the soil.

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A GREEN MANURE FERTILIZER STUDY ON NORFOLK SAND[1]

J. E. ADAMS, E. M. ROLLER,[2] AND H. M. BOGGS[3]

Bureau of Plant Industry, U. S. Department of Agriculture

Received for publication May 6, 1936

This report gives results of an experiment which is part of a general study of green manure and fertilizer problems planned to develop means of improving sandy soils of the South. The results were obtained at the South Carolina Agricultural Branch Experiment Station (the Sandhill Experiment Station), near Columbia, S. C., which is located in the Sandhill belt of the Southeast.

The sandhill area of the Southeast is shown in figure 1. That portion which lies in South Carolina varies in width from 10 to 30 miles, and comprises approximately 10 per cent of the area of the state. The soils are principally of the Norfolk and related series. The sandhill phase of Norfolk sand on which the experiment was made is of a coarse and an open texture. The native cover, following deforestation of the long-leaf pine, is principally dwarf oak, sedge, and other plants capable of growing on soil low in fertility and subject to excessive leaching. The cultivated areas are used for the production of cotton and corn; more recently peaches, grapes, dewberries, and asparagus are being grown.

REVIEW OF LITERATURE

The various symposia which have been held in recent years (20, 21, 22) have focused attention upon the pertinent relations of organic matter to soils and crops. Excellent résumés of the literature have been made by Waksman (27) on the nature of organic matter, by Thorne (25) on its relation to crop production, and by Salter (14) on the composition as related to its effectiveness.

The factors which influence the decomposition of organic matter in the soil have been investigated by Thom and Smith (24), Thom and Humfeld (23), Waksman (26), and others.

The effect of the carbon-nitrogen ratio of various organic materials and of the soil itself upon the availability of nitrogen in the soil has been investigated

[1] A contribution from the Division of Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the South Carolina Experiment Station, coöperating, at the Sandhill Experiment Station, Columbia, S. C. Credit is due Dr. J. J. Skinner, of the Bureau of Plant Industry, for advice in developing plans of the studies and for guidance in prosecuting the work, and acknowledgment is made to the Office of Forage Crops and Diseases, Bureau of Plant Industry, for their aid in the management of plats.

[2] Associate soil technologist, and assistant chemist, respectively.

[3] Assistant chemist, transferred to Food and Drug Administration, December, 1935.

by Brown and Allison (5), Doryland (7), Blair and Prince (4), Jensen (11), Salter (15), Conrad (6), and many others. The Norfolk soils are classified by Anderson and Byers (2) as yellow podzols. Waksman and Hutchings (28) point out that the organic matter of podzols is characterized by a high cellulose and hemicellulose and a low protein content which produces a ratio, for the soils mentioned, of 22.5 to 41.7. The ratio for the Norfolk sand used in this experiment is approximately 26. These soils contain charcoal due to the prevalent practice of "burning over" during the early spring season; this

FIG. 1. SANDHILL SECTION OF NORTH CAROLINA, SOUTH CAROLINA, AND GEORGIA, LYING BETWEEN THE PIEDMONT AND ATLANTIC COASTAL PLAINS BELTS

Shaded section is the sandhill section. (From U. S. Dept. Agr. Tech. Bul. 452, 1934)

contributes to the wide ratio. Heyward and Barnette (10) and Greene (9) have found this practice to be beneficial.

The lighter Norfolk soils of North Carolina are rated by Williams et al. (29) with a value of 1 to 7, the best soils being rated at 10. It is considered here that the results obtained on the Norfolk sand differ from those on the heavier types in degree rather than in general nature. The fertilizer studies of Skinner (16), Skinner and Buie (17), and Skinner et al. (18) have demonstrated the need for complete fertilizers for the production of corn and cotton,

with emphasis placed on the need for nitrogen. Bell (3) has found that the decomposition of some legumes depresses the nitrate-nitrogen content of Norfolk sand. Cowpeas and manure, however, were found to increase the nitrate content of the soil.

Funchess (8) states that the low crop yields of the South are due more to a deficiency of available plant food than to any single factor, and that "the need for nitrogen is greatest from a relative standpoint." The need for nitrogen on Norfolk sand is outstanding.

DESCRIPTION OF EXPERIMENT

The area of Norfolk sand used in this experiment was cleared in 1919.[4] It was cropped to corn during the years 1920, 1921, and 1927; the last season mentioned was the first year it was under the supervision of the State of South Carolina. Cotton was grown from 1922 to 1926, inclusive. The corn was fertilized with 400 of a 4–8–4 fertilizer[5] and side-dressed with 100 pounds of nitrate of soda per acre. The cotton received 800 pounds of fertilizer of a like formula and the same amount of nitrate of soda as for the corn.

Twenty-four plats (1/20 acre), arranged in six tiers of four each and replicated once, were used in a rotation of legumes, corn, and cotton. The tiers are paired for the sake of comparison. Legumes, i.e., soybeans, velvet beans, and cowpeas, were grown on one tier with 400 pounds of a 6–8–4[6] fertilizer and on the second tier with a like application of a 2–8–4 fertilizer. An additional plat of cowpeas, to be followed by a winter cover crop of rye and vetch, completed each of the two tiers. The hay was removed from the tier receiving the 6–8–4 fertilizer, and the stubble was turned; the full crop was turned as a green manure in early September of each year on the tier receiving a 2–8–4 fertilizer. The same fertilizer ratios were maintained on the stubble and green manuring plats for the corn and cotton grown successively in the rotation. The corn received an application of 400 pounds per acre; the cotton, 800 pounds. The year 1934 marked the end of the second round of the rotation.

METHODS

Soil samples were taken semi-annually during the experiment. These were analyzed for total carbon by the wet oxidation method of Adams (1), and for total nitrogen by the Gunning-Hibbard method, as given in the Official Methods of the Association of Official Agricultural Chemists. The pH values were determined according to the hydrogen-electrode procedure of Snyder (19).

[4] History of area furnished by Mr. J. A. Riley, Superintendent, Sandhill Experiment Station, Columbia, South Carolina.

[5] NH_3-P_2O_5-K_2O.

[6] The fertilizer ratio is N:P_2O_5:K_2O. One-third of the nitrogen was derived from nitrate of soda, one-third from sulfate of ammonia, and one-third from cottonseed meal; the phosphoric acid was from 16 per cent superphosphate; and the potash, from sulfate of potash.

YIELDS DATA

The average yields for all crops are given in table 1 for the first round (1929–1931) and the second round (1932–1934) of the rotation, as well as for the 6 years. The yield of hay in plats where the vegetation was turned under was obtained by cutting and weighing several areas 5 feet by 5 feet and returning the vegetation. The growing corn and cotton, the indicator crops, are shown in plate 1. The 6–8–4 fertilizer and stubble system has produced larger yields of all crops than has the 2–8–4 and green manure. Average

TABLE 1

Average yields, per acre, of crops grown on legume-stubble plats with 6–8–4 fertilizer and on green-manure plats with 2–8–4 fertilizer, on Norfolk sand: 1929–1931; 1932–1934; 1929–1934

SUMMER CROP AND WINTER MANAGEMENT	STUBBLE TURNED PLUS 6–8–4			GREEN MANURES TURNED PLUS 2–8–4*		
	Green Hay	Corn	Seed Cotton	Green Hay	Corn	Seed Cotton
	tons	*bushels*	*pounds*	*tons*	*bushels*	*pounds*
Soybeans, fallow:						
Ave. 1929–31	2.15	10.77	437	1.64	8.71	290
" 1932–34	2.04	11.51	688	1.53	8.83	375
" 1929–34	2.10	11.14	563	1.59	8.77	333
Velvet beans, fallow:						
Ave. 1929–31	3.68	9.49	368	2.66	8.17	227
" 1932–34	3.22	10.72	567	2.40	8.46	306
" 1929–34	3.45	10.11	468	2.53	8.32	267
Cowpeas, fallow:						
Ave. 1929–31	2.09	10.15	370	1.63	7.10	221
" 1932–34	2.49	10.94	627	2.20	8.94	322
" 1929–34	2.29	10.55	499	1.92	8.02	272
Cowpeas, rye, and vetch:						
Ave. 1929–31	2.45	11.53	564	1.98	9.22	371
" 1932–34	3.06	15.12	915	2.67	11.06	472
" 1929–34	2.76	13.33	740	2.33	10.14	422

* Legumes and corn received 400 pounds per acre; cotton, 800 pounds.

yields of seed cotton for 6 years from the former system show increases over the latter as follows: soybeans, 230 pounds; velvet beans, 201 pounds; cowpeas, 227 pounds; and cowpeas, rye, and vetch, 318 pounds. The yields of corn are consistently greater than those of the 2–8–4 and green manure plats but are not in proportion to those of cotton.

The cash values of the crops grown under the 6–8–4 and stubble system are considerably greater than those under the other, because of increased yields of corn and cotton and of the hay, which is handled as a cash crop. When a uniform basis for the cost of materials and crops produced is used, the follow-

ing increased values, per acre per year, are obtained: soybeans, $5.61; velvet beans, $7.29; cowpeas, $5.60; and cowpeas, rye, and vetch, $7.82. The high value of velvet beans in the rotation is due principally to the hay produced rather than to an unusual increase in the production of corn and cotton. An additional value should be attached to the winter cover of rye and vetch due to conservation or maintenance of nitrogen, as shown in table 2.

The pH of the plats decreased from 5.5 to 4.8 during the first round of the rotation. An application of 300 pounds of hydrated lime was made each spring of the second round to the two tiers of plats on which legumes were to be grown. This allowed the full effect to be exerted on the legumes. The pH changed from 4.8 to 5.8. The data for the second round gives the effect of lime upon the legumes for 3 years, upon corn for 2 years, and upon cotton for 1 year.

TABLE 2

Carbon and nitrogen relationships in Norfolk sand, with legume stubble turned and crops grown with a 6–8–4 fertilizer, as compared with legumes turned as green manures and crops grown with a 2–8–4 fertilizer, in a rotation of legumes, corn, and cotton

SUMMER CROP AND WINTER MANAGEMENT	STUBBLE TURNED PLUS 6–8–4 FERTILIZER						GREEN MANURES TURNED PLUS 2–8–4 FERTILIZER					
	Total C		Total N		C/N		Total C		Total N		C/N	
	1928	1934	1928	1934	1928	1934	1928	1934	1928	1934	1928	1934
	per cent	*per cent*	*per cent*	*per cent*			*per cent*	*per cent*	*per cent*	*per cent*		
Soybeans, fallow	0.54	0.40	0.020	0.018	27.0	22.2	0.51	0.36	0.020	0.017	25.5	21.2
Velvet beans, fallow	0.48	0.38	0.020	0.018	24.0	21.1	0.50	0.38	0.020	0.018	25.0	21.1
Cowpeas, fallow	0.52	0.38	0.020	0.019	26.0	20.0	0.51	0.38	0.020	0.017	25.5	22.3
Cowpeas, rye, and vetch	0.54	0.43	0.019	0.020	26.8	21.5	0.50	0.42	0.019	0.020	26.3	22.1

The average yeilds of soybeans and velvet beans were decreased during the second round, while those of cowpeas, for both systems as well as for fallow and the winter cover, were increased. Corn showed a slight but consistent increase during the second round for both systems. The gains for the cotton during the second round over those of the first averaged 263 pounds of seed cotton per acre on the 6–8–4 and stubble plats, while the increase for the other system was 84 pounds. The stubble and 6–8–4 combination is superior to the green manure and 2–8–4 management, except in the case of corn grown on the cowpea-winter fallow plats. Not only was the greatest increase in yields of both crops obtained on the cowpea-rye-vetch plats but also the greatest total yields. If there was any effect of lime on these crops during the second round, it was entirely overshadowed by the differences due to the combined fertilizer treatment and crop management.

CARBON-NITROGEN DATA

Charcoal in this soil, due to the practice of "burning over" already discussed, undoubtedly contributes to the wide carbon-nitrogen ratio. Any change in

the ratio during the course of the experiment, however, should be due to the particular treatment. Comparisons should, therefore, be valid. The type of vegetation and the coarse texture of the soil, which allows excessive leaching, give rise to organic matter of a wide ratio and low in amount. The soil appears to be analogous to a sand culture to which material of a wide carbon-nitrogen ratio has been added. The management supplying the greatest quantity of nitrogen available to the indicator crops should show increased yields. The data indicate that the extra 4 per cent of nitrogen applied to the stubble plats supplies a greater quantity of available nitrogen than does the other system of management.

The carbon and nitrogen data are given in table 2. Leighty and Shorey (12), Anderson and Byers (2), and McLean (13) have shown that the variable

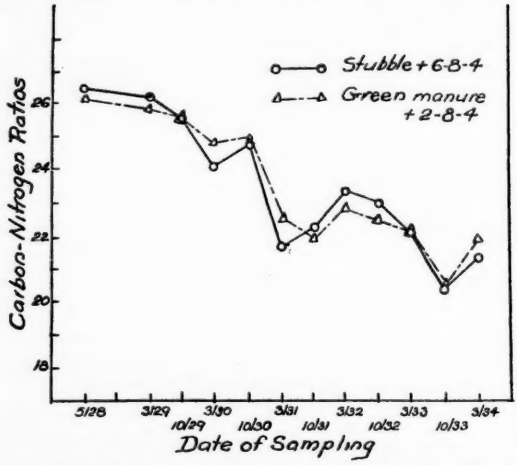

FIG. 2. CHANGE IN CARBON-NITROGEN RATIOS FOR AVERAGE OF ALL PLATS OF THE 6–8–4 AND STUBBLE SERIES VS. 2–8–4 AND GREEN MANURING SERIES, FOR ORIGINAL SAMPLES OF MAY, 1928, TO SAMPLES OF MARCH, 1934

carbon-nitrogen ratio of soils makes unreliable the use of a factor to convert organic carbon to organic matter. For this reason, and because of the presence of charcoal in the soil, the term "carbon" is used except where the term "organic matter" is used in the broadest sense.

A very appreciable loss of carbon has occurred on each of the 48 plats, irrespective of the fertilizer treatment and management. These represent from 16 to 27 per cent of the total carbon present at the beginning of the experiment. The use of rye and vetch has resulted in a smaller loss under both systems of management. There are losses of nitrogen on all plats left fallow during the winter season, the losses being slightly greater where the crops were turned as green manures. Plats on which cowpeas were followed by a winter cover show a slight gain in nitrogen for both systems of management.

The stubble and 6–8–4 system is slightly superior to the green manure and 2–8–4 system in regard to the conservation of organic carbon and nitrogen when winter fallow is practiced. When a winter cover is used these two systems are of equal value.

There has been a considerable decrease in the carbon-nitrogen ratio for each of the 48 plats. Figure 2 depicts the change in the carbon-nitrogen ratio obtained as an average of all 24 plats on which the 6–8–4 fertilizer and stubble management was used, as compared with the same number of 2–8–4 and green manure plats, irrespective of the crops grown. The data give a direct comparison of the effect of each of the systems upon the ratio, for the years 1928 to 1934, and show that both systems affect the ratio equally. It is thought that the increased yields of corn and cotton during the second round of the rotation

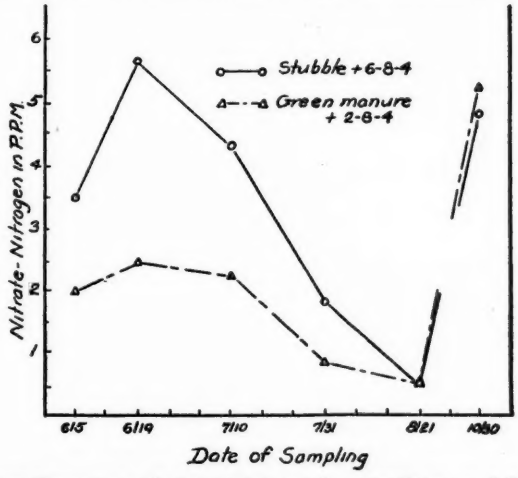

FIG. 3. NITRATE NITROGEN IN SOIL OF 6–8–4 AND STUBBLE PLATS VS. 2–8–4 AND GREEN MANURE PLATS DURING 1933

Last half of fertilizer applied June 15; stubble and green manures turned August 18

are associated, in a general way, with the decreased ratios. The reduction should result in a greater supply of nitrogen available for plant growth. The rate at which the ratio is decreasing indicates that some years will probably elapse before an optimum ration will be established.

NITRATE NITROGEN

Determinations of the nitrate nitrogen throughout the summer season of each year, for which tables are not given, show a low average content. Figure 3 shows the average nitrate nitrogen content for the season of 1933 for the 6–8–4 combination versus the 2–8–4 combination, calculated in the same way as for the carbon-nitrogen ratios. The 1933 season was chosen because leaching was not excessive. Excessive rainfall would minimize these differences.

The generally increased supply of available nitrogen due to the use of the 6–8–4 fertilizer has produced larger average yields of all crops.

RESIDUAL CARBON AND YIELDS

Figure 4 presents graphically the relations between the carbon content of the plats, at the end of the second round of the rotation, and average yields of corn and cotton for the 6 years. Tabular data are not given. Data for the plats are plotted separately rather than as averages of two replications.

The relations of yields to the residual carbon contents of the plats are masked by the influence of the nitrogen in the fertilizer and by the effect of

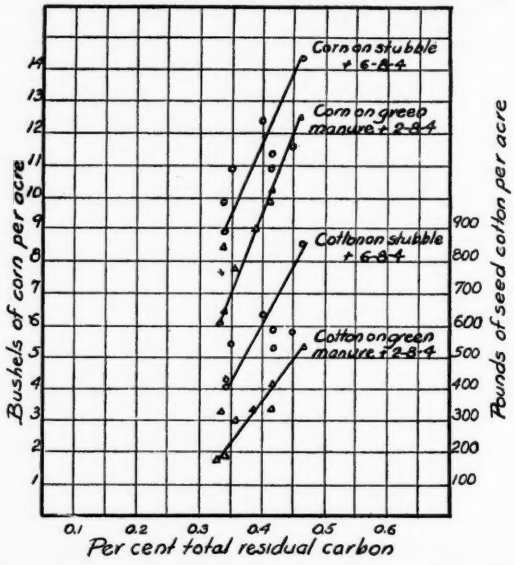

FIG. 4. RELATION OF YIELDS OF CORN AND COTTON TO RESIDUAL TOTAL CARBON CONTENT OF PLATS, WHEN GROWN ON LEGUME STUBBLE AND SUPPLEMENTED WITH 6–8–4 FERTILIZER, AS COMPARED WITH LEGUMES TURNED AS GREEN MANURE AND FERTILIZED WITH 2–8–4

the winter cover crop. There is a loose correlation between carbon and yields; it is somewhat better for the green manure and 2–8–4 system than for the stubble and 6–8–4.

SUMMARY

A comparison has been made of legumes stubble with the full crops of the legumes turned as green manures, when used in a rotation with corn and cotton. Crops grown on stubble plats were fertilized with materials forming a 6–8–4 ratio; those grown where legumes were turned received a 2–8–4 fertilizer. From data obtained on Norfolk sand, the following conclusions are drawn:

A 6–8–4 fertilizer applied to crops grown where the stubble of soybeans, velvet beans, and cowpeas had been turned produced larger yields than where the 2–8–4 fertilizer supplemented the green manures, all plats being left fallow during the winter.

Rye and vetch used as a winter cover following cowpea stubble, and also where cowpeas were turned as green manure, produced greater yields than on plats allowed to lie fallow during the winter. The higher nitrogen fertilizer-cowpea stubble-winter cover combination produced the largest yields.

The use of the stubble and 6–8–4 combination results in a greater conservation of carbon and nitrogen than the green manure and 2–8–4 combination. The two systems are practically of equal efficiency in the case where a winter cover follows both cowpea stubble and cowpeas turned as a green manure.

Yields of corn and cotton were but loosely associated with the total carbon content of the soil at the end of the second round of the rotation. This correlation was less apparent when increased quantities of nitrogen were applied in the form of commercial fertilizers.

There has been a considerable reduction in the carbon-nitrogen ratio of the soil. This is thought to be associated with an increase in the availability of the nitrogen present.

Yields were influenced more by the nitrogen of the fertilizer than by the management of the legumes. The winter cover was apparently more effective in influencing yields than was the legume green manure or legume stubble.

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PLATE 1

FIG. 1. Cotton in green manure fertilizer experiment 1933. Left, cotton following cowpea stubble turned, 800 pounds per acre of 6–8–4 fertilizer; center row, no fertilizer; right, cotton following cowpeas turned as green manure, 800 pounds per acre of 2–8–4 fertilizer.

FIG. 2. Corn in green manure fertilizer experiment 1933. Left, corn following cowpea stubble turned, 400 pounds per acre of 6–8–4 fertilizer; center row, no fertilizer; right, corn following cowpeas turned as green manure, 400 pounds per acre of 2–8–4 fertilizer.

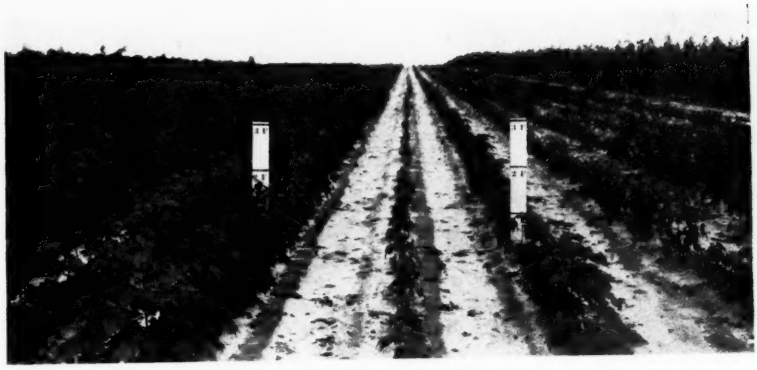

FIG. 1

FIG. 2

PHOSPHORUS NUTRITION OF CITRUS AND THE BENEFICIAL EFFECT OF ALUMINUM

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Lemon cuttings grew readily in shallow nonaerated solution cultures, but orange or grapefruit cuttings offered considerable difficulty. Omission of the phosphate from the solution for various periods of time proved beneficial, but the use of aluminum in the absence of phosphate gave the best growth.

It is possible that there is a precipitating action of aluminum with phosphorus, not only without, but within the plant and an essential need for aluminum in citrus. This paper deals with the phosphorus nutrition of citrus and its relation to aluminum.

The methods employed in growing the cuttings, in preparing the material for analysis, and in determining the total phosphorus have previously been described (2).

PREVIOUS INVESTIGATIONS

The investigations on phosphorus and aluminum have become so numerous that only the papers pertinent to the present problem will be discussed. The literature on the phosphorus relation in citrus has previously been briefly summarized (2). The essential nature of aluminum for plant growth was indicated in studies conducted by Sommer (8).

Aluminum in concentrations as low as 1 p.p.m. in solutions lacking phosphate was found by Ligon and Pierre (3) to be definitely injurious to plant growth. Aluminum depresses growth, according to Line (4), by removing phosphate or by raising the acidity but not as a result of aluminum toxicity.

By using the citrate as the source of aluminum, McLean and Gilbert (5) demonstrated that aluminum is toxic even in solutions with very low acidity (pH 6+), and that from 3 to 13 p.p.m. of aluminum constitute a stimulating dose for the plants used, toxicity appearing at about 16 p.p.m. Sideris (7) found that pineapple plants in culture solutions endured concentrations as high as 25 p.p.m. without injury.

EXPERIMENTAL

In preliminary studies, rooted lemon leafy-twig cuttings were used in 2-quart Mason jars as the containers for aerated and nonaerated culture solutions. The solutions of some cultures contained 105 p.p.m. phosphate added

as KH_2PO_4; in others the phosphate was omitted; and in still others 0, 5, 10, 15, 20, 25, and 30 p.p.m. of aluminum were added as the citrate to the phosphate-free solution. The culture solution lacking phosphate had the following composition (p.p.m.):

Na	K	Mg	Ca	NO_3	SO_4	Cl	Fe	Mn	B
7	185	54	159	789	216	10	0.1	6.2	0.5

The cuttings were grown from January 29, 1930, to December 23, 1931. At the conclusion of the experiment the cuttings showed a beneficial effect of phosphate on the growth of the tops. A too-prolonged absence of phosphorus at the higher concentrations of aluminum affected the tops in that the leaves occurred at considerable intervals on long shoots. The presence of aluminum improved the health of the root system and retarded the growth of the tops. The roots, when supplied with aluminum, were healthy white in color and grew deep down into the culture solution regardless of the factor of aeration.

In preliminary experiments, lemon seedlings also were used to test the effectiveness of aluminum on growth. The plants were grown in a nonaerated culture solution of the following composition (p.p.m.):

Na	K	Mg	Ca	NO_3	SO_4	PO_4	Cl	Fe	Mn	B
7	185	54	0	225	216	105	10	0.1	6.2	0.5

plus 0.1 p.p.m. of minor elements such as I, Br, Ti, Zn, NH_4, and Sr. Calcium citrate was added to the solution in some jars and calcium aluminate in others as a source of calcium.

The absence of calcium brought about injury to the roots, and the tops then wilted and died (pl. 1, fig. 1). The plants grown in a solution supplied with calcium as the citrate were able to produce some growth in the tops and roots, but the root growth was accompanied by injury. The use of calcium aluminate brought about a beneficial effect not only in the roots but also in the tops.

In experiments with large citrus cuttings grown for approximately one year or more, a study was made of the phosphorus content in relation to the general growth response obtained when a given culture solution was used.

Attention was first given to the phosphorus content in various portions of lemon cuttings grown in culture solutions of maintained pH. Lisbon and Eureka lemon cuttings were grown from February 24 to December 7, 1933, in nonaerated culture solutions in some of which the pH was maintained at definite levels (pH 4.5) by means of dilute nitric acid. In some solutions 105 p.p.m. phosphate was continually present; in others it was either absent at all times or was present (105 p.p.m.) for only a few days at intervals of several weeks. The culture solution lacking phosphate had the following composition (p.p.m.):

Na	K	Mg	Ca	NO_3	SO_4	Cl	Fe	Mn	B
7	185	54	159	789	216	10	0.1	6.2	0.5

Various concentrations of aluminum as the nitrate or citrate were added to some of the culture solutions.

Plate 1, figure 2, shows the growth, in the absence of aluminum, of cuttings in phosphate-free cultures and in cultures in which 105 p.p.m. phosphate was present for a few days at intervals of several weeks. The tops and roots were in a healthy condition when phosphate was present only at intervals. Although very little growth was made when phosphate was continually absent, the roots remained in good condition.

In the presence of aluminum the top growth is poorer and the root growth better in the continued absence of phosphate (pl. 1, fig. 3*A*) than when phosphate is present occasionally in the culture solution (pl. 1, fig. 3*B*). Comparison of the growth made by the cuttings in the cultures without phosphate (pl. 1, fig. 2*A* and 3*A*) indicates that aluminum has greatly improved the root system when phosphate was deficient.

Comparison of the growth made by the cuttings in the cultures containing phosphate for a few days at intervals of several weeks (pl. 1, fig. 2*B* and 3*B*) indicates that the aluminum stimulated the growth of the root system but at the apparent expense of the top growth.

Gericke (1) has directed attention to the beneficial effect to plant growth of the temporary depletion of some of the essential elements in the soil. The results for citrus have indicated that when phosphorus is temporarily absent from the culture solution the presence of aluminum is beneficial to the root growth. The concentrations of phosphate in the soil solution fluctuate from time to time, and the periods of minimum phosphate concentration may be considered of advantage to the plants in that the concentrations of other elements have an opportunity to increase.

Pierre and Stuart (6) found that the beneficial action of large phosphate additions to acid soils is partly due to a reaction of aluminum and phosphate within the plant and not entirely to the precipitation of aluminum in the soil solution.

In the absence of aluminum in the nonaerated culture solution, there were beneficial effects on the growth of the root system of Eureka lemon leafy-twig cuttings when phosphate was omitted and a poor type of root development when 105 p.p.m. phosphate was present. As shown in plate 1, figure 4B, both root systems were finely divided and dark in color, especially that grown in the high phosphate solution. The phosphate deficiency leaf symptoms were just beginning to make their appearance in the plants grown in the solutions lacking phosphate.

Other Eureka lemon cultures (pl. 1, fig. 4*A*) were grown in solutions containing 105 p.p.m. phosphate for a few days at intervals of several weeks and contained aluminum during the omission of the phosphate.

The presence of aluminum in the solution most of the time, with phosphate supplied for only a few days at long intervals, is particularly beneficial to the root system. When aluminum is present, the roots are white, coarse, and

TABLE 1

Phosphorus content, as per cent of dry matter, of various portions of lemon cuttings grown in culture solutions of maintained pH

LAB. NO.	DURATION OF THE EXPERIMENT (1933)	CULTURE SOLUTION	P CONTENT OF		
			Leaves	Shoots	Roots
		(*Lisbon lemon*)			
1	Feb. 24 to Dec. 7	pH 4.5 with HNO_3; 10 p.p.m. Al added as the nitrate; PO_4 always absent (pl. 1, fig. 3*A*)	0.0488 0.0494* 0.0599	0.0291 0.0275* 0.0369	0.0600 0.0750* 0.1250
2	Feb. 24 to Dec. 7	pH 4.5 with HNO_3; 10 p.p.m. Al added as the nitrate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks (pl. 1, fig. 3*B*)	0.0650*	0.0375*	0.1625*
		(*Eureka lemon*)			
3	April 11 to Dec. 26	pH 4.5 with HNO_3; Al always absent; 105 p.p.m. PO_4 always present (pl. 1, fig. 4*B*)	0.2500	0.2688	0.3125
4	April 11 to Dec. 26	pH 4.5 with HNO_3; Al and PO_4 always absent (pl. 1, fig. 4*B*)	0.0875	0.0431	0.0800
5	April 11 to Dec. 26	pH 4.5 with HNO_3; 10 p.p.m. Al added as the citrate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks (pl. 1, fig. 4*A*)	0.1000	0.0438	0.2000
6	April 11 to Dec. 26	pH 4.5 with HNO_3; 20 p.p.m. Al added as the citrate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks (pl. 1, fig. 4*A*)	0.0938	0.0525	0.3625
7	April 11 to Dec. 26	pH 4.5 with HNO_3; 10 p.p.m. Al added as the nitrate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks	0.1125	0.0675	0.2500
8	April 11 to Dec. 26	pH 4.5 with HNO_3; 20 p.p.m. Al added as the nitrate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks	0.1219	0.0538	0.2781
		(*Lisbon lemon*)			
9	Mar. 4 to Dec. 23	pH 4.5 with H_2SO_4; Al always absent; 105 p.p.m. PO_4 always present.	0.1750	0.1800	0.7000
10	Mar. 4 to Dec. 23	pH 4.5 with H_2SO_4; 15 p.p.m. Al added as the sulfate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks	0.0600	0.0350	0.2000
11	Mar. 4 to Dec. 23	pH 4.5 with H_2SO_4; Al always absent; 105 p.p.m. PO_4 present for a few days at intervals of several weeks.	0.0613	0.0438	0.1188

* Repetition experiments.

healthier in appearance than when aluminum is absent. At a concentration of 20 p.p.m. aluminum, whether as the citrate or the nitrate, the growth of both tops and roots is retarded. Later, these effects upon the roots will be given more detailed consideration.

In table 1 are given the percentages of phosphorus in the dry matter of the various portions of lemon cuttings. The cultures were grown in phosphate-free solutions of the following composition:

Na	K	Mg	Ca	NO_3	SO_4	Cl	Fe	Mn	B
7	185	54	159	789	216	10	0.1	6.2	0.5

To this solution were added various concentrations of aluminum in the form of the citrate, nitrate, or sulfate. Phosphate, when present, was added as potassium acid phosphate.

When 105 p.p.m. phosphate was always present in nonaerated culture solutions with lemon cuttings, the roots became gelatinous and the leaves light-veined. Table 1, numbers 3 and 9, indicate the high phosphorus concentration in leaves of such cuttings.

Excellent vegetative growth of the tops was obtained when the phosphorus content of the dry matter of the leaves was between 0.06 and 0.12 per cent. No definite evidence was obtained that the addition of aluminum to the culture solution increased the percentage of phosphorus in the dry matter of the leaves.

A concentration of 15 to 20 p.p.m. of aluminum was rather high for the production of the greatest growth. pH 4.5 is rather close to the minimum pH value that the roots will withstand. The great metabolic activity of lemon cuttings (in comparison with that of orange) permits lemon roots to change more readily the pH of the culture solution toward the more favorable higher pH values. The nature of the acid and similarly that of the alkali used in regulating the pH values of the culture solutions for the growth of citrus cuttings have an effect on the growth produced. In the presence of considerable sulfate, it is more difficult to maintain a green color in the leaves, although an increased amount of iron is of benefit.

When lemon cuttings are grown in the presence of aluminum and in the absence of phosphorus, burning of leaves may occur because of a deficiency of phosphorus. On account of the reduced top growth in the presence of considerable aluminum and in the absence of phosphorus, the leaves remain attached for a longer time than when aluminum is also absent.

In the early stages of growth the roots of Lisbon lemon cuttings in nonaerated culture solution containing at all times 105 p.p.m. phosphate, are light colored and the laterals short. Later the roots become extremely dark-colored and gelatinous. In the absence of phosphate in the culture solution until deficiency symptoms are evident in the tops, the roots have longer laterals than when 105 p.p.m. phosphate is continually present. As the phosphate deficiency becomes more acute, the roots become dark colored but re-

main alive. In the continued absence of phosphate and in the continued presence of aluminum, the plants have few but long sturdy roots which remain in a healthy condition longer than roots grown without aluminum. The presence of aluminum in the solution most of the time, with phosphate supplied for only a few days at long intervals, maintains this healthy condition of the root system in solution cultures.

The beneficial action of aluminum may be due to the slow but steady supply of phosphorus obtained from the reutilization of phosphates precipitated by the aluminum in or on the roots. The presence of aluminum in the culture solution when phosphate continually is omitted is of assistance, not only in retarding the growth of the top and thereby slowing up the injury to the leaves, but in increasing the health of the root system. This latter condition will later be seen as being of assistance in promoting the recovery of phosphorus-starved cuttings once phosphate is supplied in the culture solution.

The healthy condition of root tips of citrus cuttings grown in solution culture may be due to the essential need of aluminum for root cap production and possibly for the growth of the root tips themselves. Evidence was obtained that the production of root caps was increased by the presence of aluminum in the culture solution. The lemon cuttings, the roots of which are shown in plate 2, figure 1, were obtained from solution cultures containing aluminum. It is seen that many of the root tips are covered by root caps. These were not evident in roots of cuttings grown without aluminum in the culture solution.

The recovery of lemon cuttings from a severe phosphorus deficiency in which all except the youngest leaves had fallen was next studied in relation to the presence or absence of aluminum in the culture solution. The cultures were conducted from February 19 to December 22, 1933, and the pH of the culture solutions was maintained at 4.5 by means of dilute nitric acid. The cuttings grown with aluminum in the culture solution possessed larger root systems than those grown without it. On and after August 30, 1933, 105 p.p.m. phosphate was added for a few days at intervals of several weeks to the phosphate-deficient cultures.

The growth of lateral buds is related to the supply of phosphorus. When aluminum was present in the phosphate-deficient culture solution, the lateral buds started to grow within a few days after phosphate was supplied to the culture solution. The growth of lateral buds in the cultures that received no aluminum eventually starts and is tardily followed by root growth, but the recovery of top and root growth is markedly more rapid when aluminum is supplied. In other experiments of a similar kind it was found that recovery in the tops parallels the state of health of the roots.

It is of interest that the young terminal leaves are better able to resist the injuries that result from a phosphorus deficiency than are more mature leaves. It is known that immature leaves contain higher percentages of phosphorus than mature ones.

Lemon cuttings were grown from February 19 to June 7, 1933, in a culture

solution containing 20 p.p.m. of aluminum but no phosphate. The pH of the culture solution was not controlled and ranged between pH 5 and 7, which was more favorable to growth than pH 4.5. On June 7 the cuttings had lost all except the immature terminal leaves, and no lateral shoots had developed. From June 7 to November 9, 1933, 105 p.p.m. of phosphate was used in the

TABLE 2

Phosphorus content, as per cent of dry matter, of various portions of Lisbon lemon cuttings grown in culture solution and recovering from phosphate deficiency

LAB. NO.	DURATION OF THE EXPERIMENT	CULTURE SOLUTION	P CONTENT OF		
			Leaves	Shoots	Roots
1	July 14, 1932, to December 1, 1933	pH 7.6 on Feb. 19, 1933; Al and PO_4 always absent	0.0406	0.0213	.....
2	February 19 to November 9, 1933	pH 7 to 8; Al always absent; PO_4 absent until June 7, 1933, and subsequently present for a few days at intervals of several weeks	0.1125	0.0925	0.1625
3	February 19 to December 22, 1933	pH 4.5 with HNO_3; Al and PO_4 always absent	0.0438	0.0244	0.0506
4	February 19 to December 22, 1933	pH 4.5 with HNO_3; PO_4 always absent; 10 p.p.m. Al added as the nitrate	0.0325	0.0238	0.0456
5	February 19 to December 22, 1933	pH 4.5 with HNO_3; Al always absent; PO_4 absent until August 30, 1933, and subsequently present for a few days at intervals of several weeks	0.0450	0.0250	0.0800
6	February 19 to December 22, 1933	pH 4.5 with HNO_3; 10 p.p.m. Al added as the nitrate; PO_4 absent until August 30, 1933, and subsequently present for a few days at intervals of several weeks	0.0563	0.0263	0.3031
7	February 19 to December 22, 1933	pH 4.5 with HNO_3; Al always absent; PO_4 absent until June 7, 1933, and subsequently present for a few days at intervals of several weeks	0.0475	0.0275	0.1225
8	February 19 to December 22, 1933	pH 4.5 with HNO_3; 10 p.p.m. Al added as the nitrate; PO_4 absent until June 7, 1933, and subsequently present for a few days at intervals of several weeks	0.0613	0.0319	0.2500

culture solution for a few days at intervals of several weeks and aluminum was present when phosphate was absent from the culture solution.

At the conclusion of the experiment a multiplicity of new lateral shots was produced after phosphate was supplied, which shows that the lateral buds were still alive. Growth was very rapid, and the leaves were somewhat chlorotic.

The leaves were broad and not in a reflexed position at the petiole junction with the leaf blade. The roots showed vigor and were in excellent condition.

The percentages of phosphorus in the dry matter of the various portions of phosphate-starved and recovered lemon cuttings are shown in table 2. Extreme phosphorus deficiency symptoms were accompanied by low percentages of phosphorus in the leaves (numbers 1, 3, and 4 in table 2). The percentages of phosphorus found in the leaves of recovering plants varied from those typical (number 2 in table 2) of continually healthy lemon plants to those typical (numbers 5 and 7 in table 2) of phosphorus-starved plants. The percentage values indicate that the cuttings were thoroughly depleted of phosphorus at the time the phosphorus supply was resumed. The production of new growth in the recovering plants prevented any rapid build-up of the phosphorus concentrations in the leaves during the initial stages of recovery. The percentages of phosphorus in these recovering cuttings gives some conception of the minimum phosphorus concentrations necessary in citrus.

The percentages of phosphorus shown in table 2 are to be considered as being in equilibrium only for a particular phase of the recovery process. Data in this regard are given in table 3. All leaves analyzed were considered to be mature, and from the data obtained it is seen that the percentage of phosphorus in the original lemon leaves is subject to considerable change, depending on the phosphate content of the culture solution. In many cases of phosphate deficiency, there is a smaller percentage of phosphorus in the original than in the subsequently produced mature leaves. When the culture solution contains an abundance of phosphate, the original lemon leaves still contain the smaller percentages, although all of the percentages are on a higher level. Whenever 105 p.p.m. of phosphate was continually present in nonaerated culture solution, the root system eventually was injured, whereas with aeration such roots were dwarfed.

Excellent growth of pomelo leafy-twig cuttings as scions on Eureka lemon leafy-twig cuttings as stocks was obtained in culture solutions in which 105 p.p.m. of phosphate was present for only a few days at intervals of several weeks and the aluminum was present, if at all, only when phosphate was absent. The growth of the cuttings when aluminum was not used in the culture solution was considerably less than that produced during the same growth interval when aluminum was present.

It is possible that beneficial effects that occur in the absence of phosphate may be due, not alone to its absence, but to the appearance or solubility in the culture solution of traces of elements ordinarily removed for the most part from the solution when phosphate is present. To contribute information in this direction, the following experiment was devised. One set of lemon cuttings was grown in a nonaerated culture solution (pH 4.5) containing 105 p.p.m. phosphate. Another set of cultures was grown for 2 days in a culture solution containing 105 p.p.m. phosphate, then for 2 days in a similar solution lacking phosphate, and then again for 2 days in the original (fresh) solution

TABLE 3

Phosphorus content, as a per cent of dry matter, of various portions of lemon cuttings grown in culture solutions and injured by or recovering from phosphate deficiency

DURATION OF THE EXPERIMENT	CULTURE SOLUTION	P CONTENT OF		
		Leaves	Shoots	Roots
	(*Lisbon lemon*)			
July 19, 1932, to January 5, 1933	PO_4 always absent; 10 p.p.m. Al added as the citrate	0.0736	0.0280	.....
		0.0500†	.....	.....
		0.0569*	.....	.....
July 19, 1932, to January 5, 1933	PO_4 always absent; 20 p.p.m. Al added as the citrate	0.0563	0.0389	.....
		0.0602*	.....	.....
July 19, 1932, to January 5, 1933	105 p.p.m. PO_4 always present	0.2063	.....	.....
July 19, 1932, to January 5, 1933	105 p.p.m. PO_4 always present	0.2100	0.3000	.....
		0.1350*	.....	.....
July 15, 1932, to December 1, 1933	pH 7.3 on February 19, 1933; Al and PO_4 always absent	0.0363	0.0250	.....
July 19, 1932, to December 1, 1933	pH 7.8 on February 19, 1933; 10 p.p.m. Al added as the citrate; PO_4 always absent	0.0405	0.0500	.....
July 28, 1932, to December 1, 1933	pH 8 on February 19, 1933; 20 p.p.m. Al added as the citrate; PO_4 always absent	0.0373	0.0188	.....
		0.0398†	0.0206†	.....
July 28, 1932, to December 10, 1933	Composite of cuttings from 2 culture solutions; 10 p.p.m. Al added as the citrate, pH 7.8 on February 19, 1933; 20 p.p.m. Al added as the citrate, pH 6.2 on February 19, 1933; PO_4 absent until February 19, 1933, and subsequently 105 p.p.m. PO_4 present for a few days at intervals of several weeks; Al present when PO_4 absent	0.1100	0.0675	0.1844
July 14, 1932, to January 5, 1933	PO_4 absent	0.0500	.....	.....
July 14, 1932, to January 5, 1933	PO_4 absent	0.0619	0.0350	.....
		0.0500*	.....	.....
October 21, 1932, to July 5, 1933	PO_4 absent	0.0500	0.0250	.....
		0.0450*	.....	.....
October 21, 1932, to July 5, 1933	105 p.p.m. PO_4 always present	0.1625	0.1850	.....
		0.0865*	.....	.....
	(*Villafranca lemon*)			
February 27 to July 9, 1934	PO_4 absent	0.0675	.....	0.1025
		0.0494*	.....	.....

* Original leaves of cuttings.

† Repetition experiment.

containing 105 p.p.m. phosphate. During the absence of phosphate in the solution, the impurities in the salts from which the stock solutions are prepared had an opportunity to remain more soluble and to affect the root or top growth. Since no change in the growth of the root system was observed, the salt impurities apparently had no effect, possibly because of insufficient concentration.

In two other sets of cultures like the preceding ones, similar changes of culture solution were made, except that 10 p.p.m. of aluminum was present when phosphate was omitted. The roots of these cultures showed a definite response to the added aluminum. A different interval from 2 days or a different pH range of the culture solution would alter somewhat the degree of response obtained.

The beneficial effect of the presence of aluminum during the interval in which phosphate was omitted in nonaerated cultures in which rooted Valencia orange cuttings were grown is shown in plate 2, figure 2. The cultures were grown from March 18 to September 2, 1935. The use of aluminum has proved of distinct benefit to Valencia orange cuttings when the phosphate treatment is the same in all cultures. Growth was poorest when neither zinc nor aluminum was added; growth was best for the period investigated when aluminum was added without zinc. The use of zinc without aluminum gave better growth than when both zinc and aluminum were omitted.

Many experiments have indicated the beneficial effect of aluminum on the growth of citrus in solution cultures. Because of this fact, lemon leafy-twig cuttings were grown in the best grade "Wearever" aluminum kettles of approximately 15-liter capacity. The overlapping lids were also made of aluminum and, by means of clamps, were tightly joined with the kettles, thus effectively keeping dust out of the culture solutions. Although aluminum is known to remove copper completely from solutions, the age of these cuttings has not brought this element into a deficiency consideration as yet.

The aerated cultures were started on February 6 and were photographed on September 2, 1935. During the experiment the solution used for culture *A* (pl. 2, fig. 3) contained 105 p.p.m. phosphate continually, while that used for culture *B* (pl. 2, fig. 3) contained 105 p.p.m. phosphate for only a few days at intervals of several weeks. It was assumed that during the absence of phosphate in the culture solution of *B* there may have been sufficient aluminum and impurities in the aluminum made soluble to give an impetus to the growth of the cuttings. The roots in both cultures were healthy in appearance, those in *A* being noticeably shorter than those in *B*. In *A* the tops were much branched and the leaves were large and healthy in appearance. In *B* the shoots were mostly unbranched; many of the leaves were of small size, and a few showed a burn that is characteristic of a phosphorus deficiency. Cultures such as *B* that receive phosphate at intervals do not ordinarily show burn when grown under the same conditions in enameled pans or in glass containers instead of aluminum. This fact and the phosphorus content of leaves, shoots, and roots (numbers 5 to 8 in table 2) indicate that with aluminum containers

a shorter interval may be necessary between the periods of phosphate absorption.

SUMMARY

By means of solution cultures, a study was made of the phosphorus nutrition of citrus and its relation to aluminum. The use of calcium aluminate was beneficial not only to the roots but also to the tops of lemon seedlings.

The beneficial effect of phosphate on lemon leafy-twig cuttings was largely in the growth of the tops when the culture solution was aerated. When aluminum was present the roots were healthy and more extensive, but the tops usually were retarded in growth.

Excellent growth of citrus in solution cultures was obtained by the use of 105 p.p.m. of phosphate supplied for a few days at intervals of several weeks. Very little growth may occur in nonaerated cultures when phosphorus is deficient, although the roots remain in a healthy state. Aluminum greatly improved the root systems of Lisbon leafy-twig cuttings grown without phosphate. When phosphate was present for a few days at intervals of several weeks, aluminum benefited the root system at the apparent expense of the top growth.

Root caps were numerous when citrus roots were grown in the presence of aluminum in the culture solution.

Recovery experiments showed that lateral buds inhibited by a phosphate deficiency resumed growth when phosphate was supplied.

Extreme symptoms of phosphorus deficiency were accompanied by low percentages of phosphorus in the plant tissues.

The percentage of phosphorus in the original leaves of citrus cuttings was subject to considerable change, depending on the phosphate content of the culture solution.

Aluminum was beneficial to the early growth of Valencia orange leafy-twig cuttings whether or not zinc was added to the culture solution.

When aluminum containers were used as culture vessels, the roots were healthy in appearance and were much shorter when 105 p.p.m. phosphate was continually supplied than when the phosphate was present for only a few days at intervals of several weeks.

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PLATE 1

EFFECT OF ALUMINUM AND PHOSPHATE ON GROWTH OF LEMON PLANTS

FIG. 1. Aluminate as a better source of calcium than citrate for the growth of lemon seedlings in 2-quart Mason jars filled with nonaerated culture solution containing phosphate. Three corks to the left, seedlings grown in a culture solution lacking calcium; three corks in the center, seedlings grown in a similar solution plus 1 gm. of calcium citrate to each jar; three corks to the right, seedlings grown in a solution similar to that used for the seedlings on the extreme left plus 1 gm. of calcium aluminate (Kahlbaum) to each jar.

FIG. 2. Lisbon lemon leafy-twig cuttings grown in nonaerated culture solutions without aluminum: *A*, phosphate-free culture solution; *B*, 105 p.p.m. phosphate present for only a few days at intervals of several weeks.

FIG. 3. Lisbon lemon leafy-twig cuttings grown in nonaerated culture solutions with aluminum: *A*, phosphate-free culture solution to which 10 p.p.m. aluminum was added as the nitrate; *B*, 105 p.p.m. phosphate present for only a few days at intervals of several weeks and 10 p.p.m. aluminum added as the nitrate when phosphate was absent. Compare figures 2*A* and 3*A*; also 2*B* and 3*B*.

FIG. 4. Eureka lemon leafy-twig cuttings grown in nonaerated cultures from April 11 to December 26, 1933, in enameled pails of 9-liter capacity. The pH was maintained at 4.5 with dilute nitric acid. *A*, 105 p.p.m. phosphate present in the culture solution for a few days at intervals of several weeks; right, 10 p.p.m. aluminum added as the citrate when phosphate was absent; left, same, except 20 p.p.m. aluminum. *B*, aluminum absent: left, phosphate absent; right, 105 p.p.m. phosphate always present.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

PLATE 2

EFFECT OF ALUMINUM AND PHOSPHATE ON GROWTH OF LEMON AND ORANGE PLANTS

FIG. 1. Root caps on the roots of lemon cuttings grown in a culture solution containing no phosphate and to which 5 to 10 p.p.m. of aluminum was added as the nitrate.

FIG. 2. Beneficial effect of aluminum on the growth of Valencia orange leafy-twig cuttings in culture solutions containing 105 p.p.m. phosphate for a few days at intervals of several weeks. When phosphate was omitted the culture solution contained, left to right: no zinc, no aluminum; 1 p.p.m. zinc, no aluminum; 1 p.p.m. zinc, 1 p.p.m. aluminum; no zinc, 1 p.p.m. aluminum.

FIG. 3. Effect of continuous and intermittent phosphate in the culture solution on the growth of lemon leafy-twig cuttings in aluminum containers: *A*, 105 p.p.m. phosphate continually; *B*, 105 p.p.m. phosphate for a few days at intervals of several weeks.

FIG. 1

FIG. 2

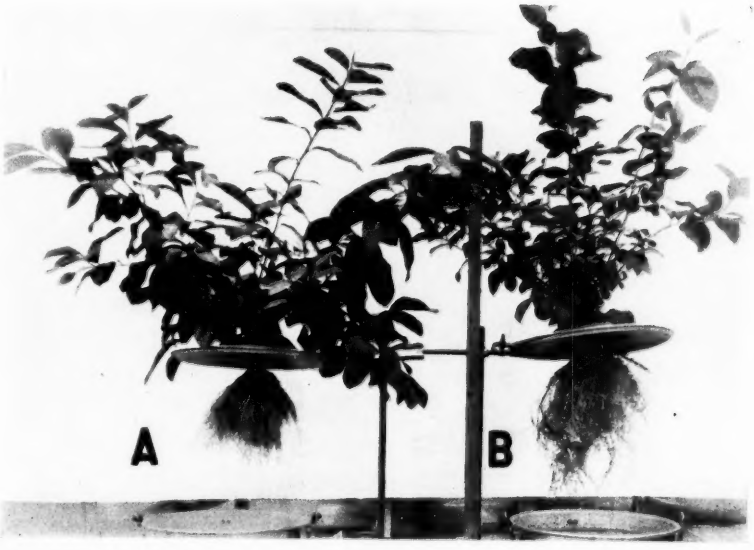

FIG. 3

UNPRODUCTIVENESS OF CERTAIN ORCHARD SOILS AS RELATED TO LEAD ARSENATE SPRAY ACCUMULATIONS[1]

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A rapidly increasing number of experiences with unsatisfactory crop growth for one to several years following the removal of old apple trees in certain orchards in central Washington have been reported in recent years. Some of these experiences are summarized by Snyder (7). The difficulty seems to occur most generally on orchard land from which trees intensively treated with insect sprays for a period of years have been removed. Headden (4) ascribed the death of many apple and pear trees in Colorado to arsenical poisoning resulting from heavy applications of arsenical sprays. Since the prevailing spray practice in the orchards of central Washington may result in considerable accumulations of lead arsenate compounds in the soil, an investigation of the relation of the concentration of these compounds in the soil to its productivity was begun in May, 1935. The preliminary results are reported in this paper.

EXPERIMENTAL PROCEDURE

A field in the Yakima Valley from which 27-year-old apple trees were removed in 1932 was selected for study. This field lay idle until the spring of 1934, when it was seeded to alfalfa with barley as a nurse crop. The alfalfa germinated poorly, and the barley plants emerged but soon turned yellow and the majority died when they reached a height of about two inches. The field was plowed in the fall and reseeded to alfalfa and barley the following spring. Again very little alfalfa emerged, and the stand of barley was exceedingly spotted, some of the spots supporting apparently normal plants. On May 14, representative samples of the surface 6-inch layer and also of the underlying 6 to 12-inch layer of soil in several apparently normal as well as poor spots were obtained for analysis. Measured quantities of one of the samples derived from the poor spots were extracted with various solutions for the purpose of finding a suitable means of determining readily soluble arsenic. One

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part of soil was mixed with five parts of cold distilled water or with a 0.1 *N* solution of various salts and allowed to stand for 4 hours with occasional shaking. The suspensions were filtered, and aliquots of the filtrates were analyzed for arsenic by the Gutzeit (1) method. The results are reported in table 1. As can be noted, the amounts of readily soluble arsenic extracted by the various solutions correlated closely with one another with the exception of those extracted by the ammonium carbonate and potassium solutions. Since flocculation of the soil is desirable to speed up filtration, the 0.1 *N* am-

TABLE 1

Influence of extracting solutions on the amount of readily soluble arsenic

EXTRACTING SOLUTION	As_2O_3
	p.p.m.
H_2O	3.5
0.1 *N* NH_4Ac	3.7
0.1 *N* NH_4NO_3	3.6
0.1 *N* $(NH_4)_2SO_4$	3.9
0.1 *N* $(NH_4)_2CO_3$	9.6
0.1 *N* KNO_3	4.1

TABLE 2

Relation of readily soluble arsenic to that soluble in hot concentrated HNO_3

FIELD	SAMPLE	DEPTH	CROP CONDITION	As_2O_3	
				Soluble in concentrated HNO_3	Soluble in cold 0.1 *N* NH_4Ac
		inches		*p.p.m.*	*p.p.m.*
1	1	0–6	poor	48.0	3.0
		6–12		17.5	0.8
	2	0–6	poor	48.6	9.8
		6–12		7.5	3.0
	3	0–6	normal	13.3	0.8
		6–12		3.2	0.3
2	4	0–6	normal	4.0	0.3
		6–12		1.6	0.1

monium acetate solution was selected for all subsequent determinations of readily soluble arsenic, one part of soil being used to five parts of solution.

RELATION OF READILY SOLUBLE ARSENIC TO ARSENIC SOLUBLE IN CONCENTRATED HNO_3

Suitable quantities of soil from two samples derived from normal spots and two samples derived from poor spots were extracted with cold 0.1 *N* ammonium

acetate and concentrated hot nitric acid, respectively, those extracted with nitric acid being digested on a steam bath for 3 hours, one part of soil being used to five parts of acid. The filtrates of the hot nitric acid extracts were diluted sufficiently to overcome interference of the nitrate ion with the arsenic determination, and aliquots of the diluted filtrates as well as of the ammonium acetate extracts were analyzed for arsenic. The results are presented in table 2.

If it is assumed that a large part of the arsenic residue in the soil was recovered by digestion in concentrated HNO_3, the data indicate the presence of considerable quantities of this material and the accumulation of the bulk of it in the surface layer. The greatest concentration of readily soluble arsenic also seemed to occur in the upper 6 inches. Moreover, the condition of the crop as observed in the field was closely related to the amount of readily soluble arsenic in the surface layer of soil.

TABLE 3

Relation of depth to readily soluble arsenic

DEPTH	As_2O_3	
	Field 1	Field 2
inches	*p.p.m.*	*p.p.m.*
0–6	3.7	7.0
6–12	1.2	2.5
12–24	1.0	0.6
24–36	0.7	1.3
36–48	0.8	0.4

AMOUNTS OF READILY SOLUBLE ARSENIC AT VARIOUS DEPTHS IN THE SOIL

Samples of soil taken at various depths from the surface down to 48 inches were obtained from two fields, field 1 being the same from which the previous samples were obtained, and field 2, one located a few miles distant where the removal of old apple trees was followed by poor growth of the subsequent crop. The amounts of readily soluble arsenic in these samples were determined, and the results are given in table 3. The data show that in both soils the greatest concentration of readily soluble arsenic occurred in the surface 6-inch layer and decreased greatly with depth.

RELATION OF READILY SOLUBLE ARSENIC AND TOTAL WATER-SOLUBLE SALT CONTENT TO CROP BEHAVIOR

Samples of soil taken from the surface 6 inches and from a depth of 6 to 12 inches were obtained from a number of fields in the Yakima Valley where old fruit trees had been removed and where subsequent crop growth indicated various degrees of unproductiveness. One field which has never been in

orchard was included as a control. The texture of the soils ranged from fine sandy loam to silt loam, and all the fields selected were free from abnormal

TABLE 4

Relation of readily soluble arsenic and total water-soluble salt content to crop behavior

FIELD NUMBER	CROP HISTORY	CROP	CONDITION OF CROP	DEPTH	TOTAL WATER-SOLUBLE SALTS	READILY SOLUBLE As_2O_3
				inches	*p.p.m.*	*p.p.m.*
1	Field in alfalfa for many years, never planted to orchard	Barley and alfalfa	Very good	0–6	205	trace
				6–12	188	trace
2	Apple trees until 15 years ago. No lead arsenate spray since. Peach and prune trees until removed a few years ago. Seeded in 1935	Barley and alfalfa	Good	0–6	284	0.6
3	Old apple trees removed in 1932, 6 in. of surface removed in leveling	Alfalfa	Good	0–6	176	0.1
4	Jonathan apple trees removed in 1932. Amount of lead arsenate spray about one-half usual amount. Alfalfa successful in 1932. Reseeded in 1935	Alfalfa	Good	0–6	157	1.1
				6–12	195	1.0
5	Old apple trees removed in 1933. Oats and alfalfa failure in 1934. Reseeded in 1935	Oats and alfalfa	Very poor	0–6	351	8.2
				6–12	314	5.2
6	Apple trees removed in 1934 after the orchard was neglected and little lead arsenate spray used for 5 years. Strip plowed in 1934 and seeded in 1935	Barley and alfalfa	Fair	0–6	237	1.5
				6–12	247	1.9
	Strip plowed in 1935, seeded in 1935	Barley and alfalfa	Very poor	0–6	527	16.3
				6–12	752	10.4
	Strip along head ditch filled after trees were removed. Seeded in 1935	Barley and alfalfa	Good	0–6	271	1.1
				6–12	258	0.1
7	Jonathan apple trees 32 years old removed in 1933, sloping area. Seeding of alfalfa in 1934 failed. Reseeded in 1935	Alfalfa	Poor	0–6	176	4.5
				6–12	166	3.0
	Area at bottom of slope. Seeded in 1935	Alfalfa	Very poor	0–6	166	12.5
				6–12	202	4.2
8	Old Bartlett pear trees removed in 1933. Alfalfa seeded in 1934, fair results. Reseeded in 1935	Alfalfa	Good	0–6	237	1.9
				6–12	174	0.4
9	Old apple trees removed in 1933. Seeding of barley in 1934 failed. Reseeding in 1935 failed. Clover was then tried	Clover	Fair	0–6	202	2.6
				6–12	189	1.1
	Releveled area part of surface soil removed. Seeded in 1935	Clover	Fair	0–6		3.2
				6–12		2.2

alkali salt accumulations. As a further check in this regard the total soluble salt content of the samples was determined by means of the Wheatstone bridge (2). As previously, the readily soluble arsenic content of the 0.1 *N* NH_4Ac

extracts was determined. The results are recorded in table 4 together with a brief sketch of the previous cropping history, a notation of the crop growing at the time the soil samples were obtained, and the condition of this crop.

The data reveal that the concentration of water-soluble salts was not abnormally high in any of the soils and that there was a close relationship between the behavior of the crop and the readily soluble arsenic content in the surface foot of soil.

When the concentration of readily soluble arsenic in the upper 6-inch layer exceeded 2 p.p.m., marked damage to normal growth of crops resulted. Barley and alfalfa seemed to suffer severe injury or even death when the concentration of readily soluble arsenic in the surface 6 inches of soil exceeded 3 p.p.m. It is noteworthy also that in general the greatest concentration of readily soluble arsenic occurred in the surface 6-inch layer, which is the plowed layer and the layer in which the root system of young plants develops. In many of the fields where young plants suffered it was not unusual to find normal, luxuriant, old alfalfa plants which were there before the trees were removed and had escaped the action of the plow. The fact that the root system of the old plants was established beneath the layer in which the spray residue was concentrated may explain their normal appearance.

EFFECT OF DIFFERENT SOILS AND SOIL TREATMENTS ON THE BEHAVIOR OF BARLEY

Suitable amounts of soil were obtained from the surface 6 inches of representative areas in each of three fields in the Yakima Valley. The field represented by soil 1, which never was in orchard, is productive. Three attempts to grow barley or alfalfa on the field represented by soil 2 failed following the removal of apple trees in 1932. Likewise the two attempts to obtain a stand of alfalfa with barley as a nurse crop in the field represented by soil 3 failed following the removal of the apple trees in the spring of 1934.

The bulk samples of these soils were thoroughly mixed for use in pot culture experiments in the greenhouse with the view to determining the effect of additions of various salts on the toxic effect of the soils and to ascertaining more specifically the cause of the toxicity. Analyses were made for soil reaction, for total water-soluble salts, for arsenic soluble in both hot concentrated HNO_3 and cold 0.1 *N* NH_4Ac, and for lead soluble in both hot concentrated HNO_3 and cold 0.1 *N* NH_4NO_3. The procedure for preparing the soil extracts for lead determinations was identical with that for arsenic. After proper checking with the sodium sulfite method, the diphenylthiocarbazone method (8), because of its adaptability for determinations of small quantities, was employed for lead analysis of suitable aliquots of the filtrates of the soil extracts. Ammonium acetate was replaced by ammonium nitrate as an extracting agent because of the desirability of having the lead compounds in the nitrate form for this method. The results of the various analyses are given in table 5.

As may be noted from the data in this table, the total soluble salt content

was not abnormally high in any of the soils, but the reaction of soil 3, which had a pH value of 8.10, suggests the presence of some black alkali salts. This apparently enhanced the solubility of both arsenic and lead, particularly the former. The contents of arsenic and lead soluble in hot concentrated HNO_3

TABLE 5

Reactions, soluble salt content, and contents of lead and arsenic of soils obtained from three different fields

SOIL NUMBER	pH VALUES*	WATER-SOLUBLE SALTS	As_2O_3 SOLUBLE IN HOT HNO_3	As_2O_3 SOLUBLE IN COLD 0.1 *N* NH_4Ac	PbO SOLUBLE IN HOT HNO_3	PbO SOLUBLE IN COLD 0.1 *N* NH_4NO_3
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	6.75	108	trace	trace	4.95	0.07
2	7.40	202	44.0	3.7	64.6	0.24
3	8.10	315	41.0	7.0	49.6	0.24

* Determined by means of the glass electrode.

TABLE 6

*Effect of different soils and soil treatments on the yield and behavior of barley**

SOIL NUMBER	DETERMINATION	CONTROL	10 M.E. $CaCO_3$	50 M.E. $CaCO_3$	10 M.E. $CaSO_4$	50 M.E. $CaSO_4$	10 M.E. $FeSO_4$	50 M.E. $FeSO_4$
1	Dry wt. of tops.....*gm.*	4.67	4.40	6.35	4.40	4.17	4.41	5.84
	PbO in tops.....*p.p.m.*	none	none	none	none	none	none	none
	As_2O_3 in tops.....*p.p.m.*	trace	trace	trace	trace	trace	trace	trace
	Dry wt. of roots.....*gm.*	0.86	1.06	2.67	1.25	2.33	1.52	1.70
	PbO in roots.....*p.p.m.*	none	trace	trace	trace	trace	none	trace
	As_2O_3 in roots.....*p.p.m.*	trace	trace	trace	trace	trace	trace	trace
2	Dry wt. of tops.....*gm.*	1.00	1.73	2.05	2.49	3.19	3.40	3.97
	PbO in tops.....*p.p.m.*	trace	trace	trace	trace	trace	trace	trace
	As_2O_3 in tops.....*p.p.m.*	10.01	2.54	5.78	4.01	2.51	2.52	1.48
	Dry wt. of roots.....*gm.*	0.53	0.65	0.97	1.13	1.31	1.18	1.79
	PbO in roots.....*p.p.m.*	662.00	392.00	354.00	315.00	214.00	352.00	208.00
	As_2O_3 in roots.....*p.p.m.*	788.00	472.00	112.50	91.50	87.50	89.60	30.50
3	Dry wt. of tops.....*gm.*	0.57	1.03	0.54	0.76	0.60	2.02	4.28
	PbO in tops.....*p.p.m.*	trace	trace	trace	trace	trace	trace	trace
	As_2O_3 in tops.....*p.p.m.*	17.50	7.76	16.75	9.75	9.92	9.34	6.55
	Dry wt. of roots.....*gm.*	0.21	0.42	0.10	0.34	0.37	1.07	1.67
	PbO in roots.....*p.p.m.*	756.00	554.00	551.00	387.00	354.00	275.00	112.50
	As_2O_3 in roots.....*p.p.m.*	1,640.00	287.00	1,540.00	239.00	198.00	102.00	72.00

* The yields of tops and roots are those from one pot containing six plants.

were less in soil 3 than in soil 2; whereas the readily soluble arsenic content was greater in the former than in the latter and both contained equal amounts of readily soluble lead. Headden (4) observed similar effects of alkali salts on the solubility of arsenic in Colorado soils. Soil 1, which is productive and never received any lead arsenate spray, was practically free from arsenic and

contained only a very small amount of lead. These facts should be significant in connection with the toxic effects on crops produced on these soils.

Duplicate 2-kgm. portions of soil from the bulk samples of these three fields were mixed with various salts selected in an attempt to reduce the solubility of the accumulated arsenic compounds in the soil. The salts, which were applied on the basis of milliequivalents per 100 gm. of dry soil, were incorporated by thorough mixing. The soils were then placed in pots and seeded to barley. The plants in each pot were thinned to six a few days after they emerged. When the crop reached the blooming stage of maturity, the tops and roots were harvested separately, dried, and weighed. Samples of oven-dried top and root material were digested with concentrated 1:1 HCl and $KClO_3$ according to the method given by Scott (5), and determinations were made for arsenic and lead on aliquots of the filtrates of the digests. The analytical results together with the treatments and yields of dry matter from one pot in each treatment are recorded in table 6.

The condition of the barley plants produced on soil 1 was normal in every respect and, as noted from the data in table 6, was not greatly affected by the various salt treatments. The plants on the control pots containing the other two soils developed leaves with yellow tips soon after emergence, some of the affected leaves drying up completely later. These plants never passed through the shooting and heading stages and barely managed to survive. The toxic symptoms of the plants produced in the greenhouse were identical with those observed on plants in the affected fields. These symptoms together with the extremely low yield of dry matter produced are ample evidence of a distinctly toxic condition in these soils. The severity of the toxic effects in soil 2 was notably ameliorated as a result of treatments with $CaCO_3$, $CaSO_4$, and $FeSO_4$, especially the last. In soil 3, in which the toxic effect appeared to be greater than in soil 2, the treatments with $CaCO_3$ and $CaSO_4$ produced little or no beneficial effect. Both treatments with $FeSO_4$, however, proved to be distinctly beneficial. It is possible that the beneficial effects on soils 2 and 3 resulting from the treatments with calcium and iron salts, especially the latter, were caused by conversion of some of the readily soluble arsenic to less soluble forms.

When the data in table 5 are considered, it is noteworthy that in general the yield of tops on the control soils was inversely proportional to the amounts of readily soluble arsenic in the soil and the arsenic contents of the aerial parts of the plants. It is noted also that the arsenic content of the roots of the affected plants was very high.

Although the amounts of readily soluble lead in the affected soils were very small, nevertheless the roots of the barley plants produced on these soils were capable of absorbing large quantities. Evidently only traces of the absorbed lead were translocated to the aerial parts. A similar localization of lead in plants was observed by Hammet (3), who concluded that increasing concentrations of soluble lead in the nutrient medium result in proportional retarda-

tion of the root growth. Since the concentration of readily soluble lead in the soils indicated in table 6 was low, it would seem that the toxicity produced by the accumulation of lead arsenate in the soil was caused chiefly by excessive concentrations of readily soluble arsenic. An attempt to clarify this point further was made by means of the following experiment.

EFFECT OF DIFFERENT CONCENTRATIONS OF ARSENIOUS ACID, LEAD NITRATE, AND LEAD ARSENATE ON THE BEHAVIOR OF BARLEY

A number of 2-kgm. samples of dry soil were taken from the supply of soil 1 used in the preceding experiment. One series of samples was thoroughly mixed with various amounts of arsenious acid together with a sufficient quantity of ammonium nitrate to make the nitrogen content equivalent to that contained in the lead nitrate required to supply the equivalent of 50 p.p.m. PbO. Another series of samples was thoroughly mixed with various amounts of lead nitrate. In addition to these treatments duplicate samples received 50 p.p.m. lead arsenate and the same quantity of ammonium nitrate as was added to the arsenious acid treatments. Two additional samples were used as controls but to make them comparable to the others with respect to nitrogen they were given the same amount of ammonium nitrate as the arsenious acid treated samples. The soils were placed in pots, and enough water was added to adjust the moisture content to approximately the normal moisture capacity described by Shaw (6). The pots were kept for 3 days in this condition to allow time for fixation and adjustment of the added compounds. Soil samples were then obtained for readily soluble arsenic and lead determinations, and the pots, after being planted to barley, were moved to the greenhouse. Two months later when the plants were well in the shooting stage of development samples consisting of a core of soil taken from the center of each pot were obtained for analysis. The soil treatments together with the analytical results and a brief description of the condition of the plants are recorded in table 7. The condition of the plants at the age of 10 weeks is illustrated in plate 1.

Although highly soluble arsenic and lead compounds were used in this experiment, it was found that on account of fixation by the soil only very small amounts could be recovered in readily soluble form at the end of 3 days. Determinations of readily soluble arsenic and lead made on a few samples 2 hours after they were treated yielded similar amounts, indicating that the fixation process took place rapidly. From the results obtained from the analyses of the soil samples taken at the shooting stage of the barley, it appears that the concentration of readily soluble arsenic and lead did not change greatly during the course of the experiment. In general the concentration of readily soluble arsenic had a tendency to increase slightly, but that of lead, which was already very low at planting time, suffered a further reduction.

The behavior of the plants demonstrated clearly that readily soluble arsenic in certain concentrations is toxic to barley. The toxic symptoms were identi-

cal with those manifested by the barley in the affected fields and in the preceding plant culture experiment. Moreover, the degree of toxicity exhibited

TABLE 7

Effect of soil treatments with different amounts of arsenious acid, lead nitrate, and lead arsenate on the behavior of barley

NUMBER	TREATMENT	READILY SOLUBLE ARSENIC IN P.P.M. OF As_2O_3		READILY SOLUBLE LEAD IN P.P.M. PbO		CONDITION OF PLANTS
		Planting time	Stooling stage	Planting time	Stooling stage	
1 & 2	NH_4NO_3*	trace	trace	0.07	trace	Normal, vigorous plants
3 & 4	50 p.p.m. lead arsenate	0.04	0.10	0.06	0.03	Normal seedlings, very slight symptoms of toxicity at shooting stage
	Arsenious acid					
5	50 p.p.m. As_2O_3*	trace	0.20	....	....	Normal seedlings, very slight symptoms of toxicity at shooting stage
6	60 p.p.m. As_2O_3*	0.10	0.50	....	....	Normal seedlings, slight yellowing of leaf tips later, slight symptoms of toxicity at shooting stage
7	120 p.p.m. As_2O_3*	1.00	2.00	....	....	Normal seedlings, some yellowing of leaf tips later with distinct signs of toxicity, distinct toxicity at shooting stage
8	180 p.p.m. As_2O_3*	5.00	7.00	....	....	Seedlings with yellow tips, stunted growth, leaves drying, plants failed to pass through shooting stage
9	240 p.p.m. As_2O_3*	25.00	20.00	....	....	Seedlings with yellow tips, no appreciable growth, plant barely survived
	Lead nitrate					
10	50 p.p.m. PbO	....	....	0.05	0.02	Normal, vigorous plants
11	60 p.p.m. PbO	....	....	0.10	0.05	Normal, vigorous plants
12	120 p.p.m. PbO	....	....	0.16	0.05	Normal, vigorous plants
13	180 p.p.m. PbO	....	....	0.21	0.07	Normal, vigorous plants
14	240 p.p.m. PbO	....	....	0.37	0.14	Normal plants, vigorous growth but slight tendency to yellowing of lower leaves during shooting stage

* Sufficient ammonium nitrate was added to make the nitrogen content equivalent to the amount of lead nitrate supplying the equivalent of 50 p.p.m. PbO.

by the plants in this experiment was proportional to the concentration of readily soluble arsenic in a similar manner to that observed in the affected

fields. No marked toxic effects were evident when the concentration of readily soluble arsenic in the soil was less than 1 p.p.m., but the toxic action was severe where the concentration was 5 p.p.m. or more.

It is noteworthy that the amounts of soluble lead in the lead nitrate treated soils were exceedingly small. No distinct symptoms of toxic effects were produced by this compound although the growth of the barley was retarded, especially on the soils receiving 60 and 120 p.p.m. of PbO as lead nitrate respectively. Possibly concentrations exceeding 240 p.p.m. PbO might have shown toxic symptoms.

When the results of these experiments are considered as a whole, it appears that one of the chief causes of the unproductiveness of the orchard soils investigated is the excessive concentration of readily soluble arsenic which resulted from the arsenate spray accumulations. It is possible also that readily soluble lead, if liberated in sufficient concentrations from the accumulated spray compound in the soil, may be a contributing factor. Probably the exact concentration of each of these substances that will produce toxic effects on crops varies considerably, depending upon the kind of crop grown and the inherent characteristics of different soil types. Investigations of the soluble arsenic and lead tolerances of various crops on different soil types and of methods of overcoming the toxic effects of these substances are in progress and will be reported later.

SUMMARY

The cause of the unproductiveness of certain soils in the Yakima Valley from which old apple trees have been removed in recent years was investigated.

Soil samples obtained from the surface 6 inches of a number of unproductive fields contained from 4.5 to 12.5 p.p.m. of readily soluble arsenic calculated as As_2O_3. The poor condition of young alfalfa and barley in the affected fields seemed to be roughly in proportion to the concentration of readily soluble arsenic in the surface soil.

The symptoms of toxicity of barley grown in the greenhouse in pots filled with untreated soil taken from the surface 6 inches in two unproductive fields were identical with those observed in the field. The arsenic content of samples of tops and roots of this barley harvested at the blooming stage of maturity ranged from 10.01 to 17.50 p.p.m. of As_2O_3 in the tops, and from 788 to 1640 p.p.m. in the roots. Only traces of PbO were found in the tops; whereas in the roots the amounts ranged from 662.50 to 756 p.p.m.

Samples of soil free from accumulations of readily soluble arsenic and lead, and treated with various amounts of arsenious acid and lead nitrate, respectively, were used for greenhouse culture work. The toxic symptoms of the barley plants growing in samples containing readily soluble arsenic equivalent to 5 p.p.m. or more of As_2O_3 at planting time were identical with those observed on barley in the affected fields. Retardation of growth occurred, though no definite toxic effects on barley seemed to result in soil samples receiving more than 50 p.p.m. of lead nitrate.

The results as a whole indicated that one of the chief causes of the unproductiveness of the orchard soils investigated appears to be the excessive concentration of soluble arsenic resulting from lead arsenate spray accumulations in the surface soil. Soluble lead, if liberated in sufficient concentration from the accumulated spray compounds in the soil, may be a contributing factor.

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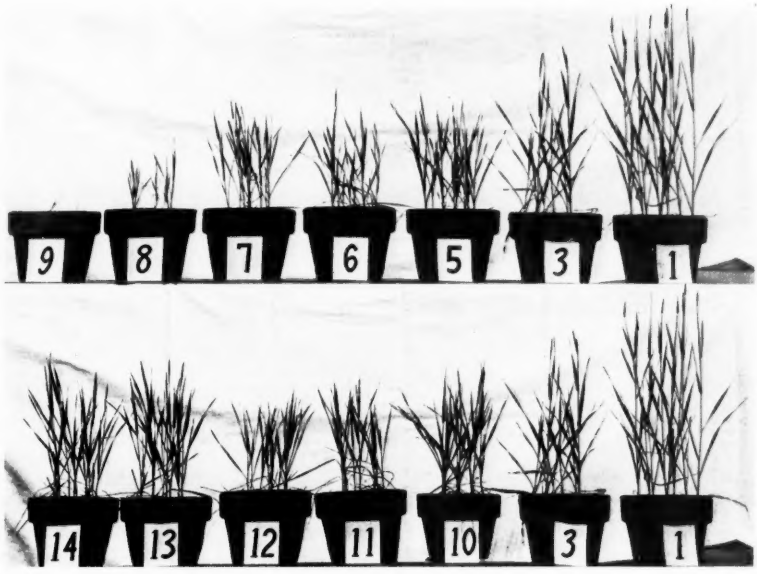
PLATE 1

EFFECT OF DIFFERENT CONCENTRATIONS OF LEAD ARSENATE, ARSENIOUS ACID, AND LEAD NITRATE ON BARLEY PLANTS

1, check; 3, 50 p.p.m. lead arsenate; 5, 50 p.p.m. As_2O_3; 6, 60 p.p.m. As_2O_3; 7, 120 p.p.m. As_2O_3; 8, 180 p.p.m. As_2O_3; 9, 240 p.p.m. As_2O_3; 10, 50 p.p.m. PbO, 11, 60 p.p.m. PbO; 12, 120 p.p.m. PbO; 13, 180 p.p.m. PbO; 14, 240 p.p.m. PbO.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THE DILATOMETER METHOD AS AN INDIRECT MEANS OF DETERMINING THE PERMANENT WILTING POINT OF SOILS[1]

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Of all the moisture constants that are usually determined, the wilting point has the most meaning and practical importance. Probably the main reason it has not been employed heretofore as much as it should in the examination of soils and in moisture studies is that the present direct method is laborious and time-consuming. There is, therefore, a great need for a practical, rapid, and accurate method for determining the wilting point of soils.

The dilatometer method gives promise of being a satisfactory indirect means for determining the wilting point of soils (1). It is the purpose of this paper, therefore, to describe this method, its principle and technique, and to show how the results obtained by it compare with those obtained by the direct method.

PRINCIPLE OF THE METHOD

The principle of the dilatometer method in determining the wilting point of soils is based upon the fact that when wet soils are super-cooled to −1.0°C. and are caused to freeze, a certain amount of the water contained in the soil fails to freeze, the amount depending upon the kind of soil. The amount of water failing to freeze at −1.0°C. is the same as that at which plants permanently wilt as determined by the direct method. The amount of water freezing is measured on the principle of expansion. According to the dilatometer used, 1 cc. of water at 0°C. expands 0.1 cc. upon freezing. When the total amount of water present is known, the amount of water that freezes or fails to freeze can thus be readily calculated.

PROCEDURE OF THE METHOD

The following materials are required for making a wilting point determination by the dilatometer method:

Dilatometer (pl. 1) having a 60 cc. capacity bulb and a 1.1 cc. stem graduated into 0.01 cc.
Small cork stoppers.
Ligroin of high boiling point.
Thermometer which has accurate reading between 0 and −10°C.

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[1] Published by permission of the director. Journal article No. 267.

A standard arrangement on the water faucet to produce suction force by the running water.
A 10-cc. and a 5-cc. pipette.
An accurate balance.
Ice and salt.
Two baths, made by putting a small crock inside a larger one, insulating the space between them with rock wool and making connections water tight.
Tallow.
Alcohol for drying the dilatometer with the aid of the suction force.

The procedure for making a wilting point determination by means of the dilatometer method consists of placing into the dilatometer by means of a small funnel 20 gm. of air-dry soil which has been passed through a 2-mm. sieve. To sand, 5 cc. of distilled water is added by means of an accurate 5-cc. pipette. To loam or clay loam, 10 cc. of water is added. If the soil is a very heavy clay or one that contains a very high content of organic matter, 5 cc. of water is first added to the dilatometer before the soil is poured in, and then 5 cc. is added on top of the soil after it is poured in. This procedure usually insures a complete wetting of the entire soil mass without stirring. After the soil is allowed to stand for about 20 minutes to absorb the water, the dilatometer is filled half full with ligroin, stoppered, and the stem is connected to the suction force at the water faucet for a few seconds to remove the air from the soil. Care must be taken not to turn on a too strong suction force which will suck away the ligroin and water. Likewise, in order to prevent the water from backing up, the suction force must be disconnected at the stem of the dilatometer and not by turning off the faucet. Sometimes it may be necessary to stir the soil. In this case the stirring is done with a thin sturdy wire, and then that portion of the wire which touches the soil is cut off by a pair of plyers and left in the dilatometer. When this procedure of suctioning and stirring the soil is followed, no loss of water takes place.

After the soil is freed of air, the dilatometer is filled with ligroin and stoppered with the cork stopper, the outside of which has been smeared with tallow to prevent any absorption of water. Whenever necessary, additional ligroin is added to the dilatometer through the stem. By holding the stem at a slight angle and adding the ligroin in small drops, the formation of bubbles in the stem is easily avoided. An attempt should always be made to maintain the level of the ligroin column near the zero point so that when the water freezes and expands, the column will not be beyond the scale.

The dilatometer is then placed in the cold bath, having a temperature of exactly -1.0°C., and left until it attains the temperature of the bath, as indicated by the ligroin column's becoming constant. As the temperature of the bath does not remain uniform throughout without frequent stirring, an effort is made to stir the mixture almost continuously. An excellent plan is to put into the bath about 6 dilatometers, or as many as it will hold, and then, by holding the stems together at the top, move them up and down in the cold mixture. This procedure not only serves to stir the mixture and keep its temperature uniform, but also helps the dilatometers to attain the temperature

of the bath faster. When the dilatometers are moved in the cold mixture, some soils may solidify prematurely. In such a case the dilatometer is taken out and warmed and the position of the soil is moved slightly by gentle tapping.

When the ligroin column in the stem has become constant after several readings, the dilatometers are placed in a separate bath having a temperature of about −10.0°C. The dilatometers are left in this bath for about 25 minutes in order that all the free water in the soil may freeze, as at the temperature of −1.0°C. it freezes very slowly and incompletely. The dilatometers are then transferred to the original bath of −1.0°C. and left in this temperature with almost continuous stirring until they attain the temperature of the bath and the ligroin column becomes constant. The excess water frozen at −10°C. over that frozen at −1.0°C. remelts quickly at the latter temperature, as indicated by the descent of the column after it reaches a certain peak, and only that portion of the water on which the soil exerts an influence remains frozen at −1.0°C. In this manner the amount of water that remains frozen at −1.0°C. becomes absolute and accurate.

The final reading, after freezing, is recorded, and from this reading is subtracted the first reading before freezing; the difference is the number of cubic centimeters of the added water that froze. By subtracting this latter amount from the amount of water that was added to the soil, one obtains the quantity of water that failed to freeze. To the latter is added also the hygroscopic water. This total amount of water is divided by the amount of soil used on the oven-dry basis, which gives the total percentage of water that failed to freeze at −1.0°C., which is considered to be the permanent wilting point of the soil.

This method is simple and very rapid. After the soil is prepared in the dilatometer it takes only about one hour to obtain the final dilatometer reading from which the wilting point is calculated. Since the dilatometer is small in volume, as many as eight dilatometers can be placed in a bath 7 inches in diameter; consequently, determinations on as many as eight soils can be run at the same time. It is possible to determine the wilting point of as many as sixteen soils in a single day.

The method is also accurate, as it gives reproducible results to within 1 per cent. The following potential sources of error, however, should be guarded against:

Premature solidification: This is easily recognized and easily remedied. It occurs in only a few soils.

Failure of all the free water to freeze at −10°C.: If the soils are left at −10°C. for about 25 minutes, all the free water will freeze.

Failure to maintain a uniform temperature in the bath: With the plan suggested, the temperature easily can be maintained uniform.

Failure to expel all the air bubbles in the soil: Probably this is the greatest source of error, especially in some particular soils which have a tendency to produce bubbles when they come in contact with the water and the ligroin. With care, however, the suction method suggested can expel all the air bubbles.

EXPERIMENTAL

In order to ascertain whether the dilatometer method as described can actually determine the wilting point of soils, a comparison was made of this method

TABLE 1

A comparison of the permanent wilting point as obtained by the direct method and by the dilatometer method

SOIL DESIGNATION	NAME OF SOIL	ADDED WATER FAILED TO FREEZE	HYGROSCOPIC WATER	TOTAL WATER FAILED TO FREEZE OR PERMANENT WILTING POINT BY DILATOMETER METHOD	PERMANENT WILTING POINT BY DIRECT METHOD*
		per cent	*per cent*	*per cent*	*per cent*
CL	Columbia silt loam	6.35	2.27	8.62	8.61
OS	Delano silt loam	3.75	1.35	5.10	4.17
FAL	Farwell silt loam	10.55	3.84	14.39	14.14
FS	Fresno sandy loam	2.60	0.67	3.27	3.05
G	Columbia silt loam	5.65	1.32	6.97	6.69
H	Stockton clay adobe	7.00	2.31	9.31	9.28
HS	Harford fine sandy	4.50	0.77	5.27	3.99
L	Columbia sand	6.65	1.13	7.78	5.53
J	San Joaquin loam	4.60	1.07	5.67	6.34
K	Sierra sandy loam	4.65	1.00	5.65	5.09
L	Yolo fine sand loam	7.35	2.21	9.56	9.11
M	Placentia loam	4.10	0.91	5.01	3.70
MG	Madera and Gridley loam	9.50	2.11	11.61	10.25
N	Okley fine sand	1.25	0.23	1.48	1.33
OC	Brockton clay	9.70	1.65	11.35	11.55
OL	Wooster silt loam	7.00	1.24	8.24	6.12
OS	Plainfield fine sand	1.50	0.29	1.79	1.36
S	Yolo silt loam	8.05	2.38	10.43	10.13
TL	Tehama loam	5.25	1.00	6.25	4.51
V	Catherine Loam	15.20	4.55	19.75	19.03
Y	Yolo fine sandy loam	6.90	2.21	9.11	8.93
YC	Yolo clay	12.2	3.67	15.87	13.98
AK_2	Aiken clay loam	13.30	3.10	16.40	20.75
YS	Yuma sand	2.95	0.78	3.73	3.17
Z	Brazito fine sand	1.25	0.40	1.65	1.58
24	San Joaquin sandy loam	4.32	0.88	5.20	3.93
25	Madera sandy loam	4.20	0.99	5.19	3.62
1	Davidson clay loam B	13.4	2.30	15.70	
2	McKenzie clay A	16.40	7.21	23.61	
3	Houston clay A	14.65	6.26	20.91	
4	Catalpa clay B	13.55	3.20	16.75	
5	Oktibbeha clay B	22.35	5.77	28.12	
6	Muck 1	53.70	15.20	68.90	
7	Muck 2	54.7	15.70	70.40	
8	Muck 3	54.0	14.60	68.60	

* Determined by Dr. F. J. Veihmeyer of the University of California.

with the direct method in which the plant was used as an indicator.[2] The results are shown in table 1.

An examination of the data reveals a remarkably close agreement between the wilting point as obtained by the two methods. It will be noted that in the majority of the soils the agreement is within 1 per cent. The only case in which the disagreement is considerable, 4.35 per cent, is in the Aiken clay loam.

DISCUSSION

A thorough consideration reveals no fundamental reason why the dilatometer method could not determine the wilting point because the wilting point represents a definite phase in the soil moisture range at which the pulling forces of the soil for the water commence to exceed the pulling forces of the plant for the same water. Now the pulling forces of the soil for the water are also exerted against the forces of crystallization or ice formation when the water freezes. At the temperature of $-1.0°$C., that portion of the water which is immediately influenced by the surface forces of the soil does not freeze and, if it freezes at lower temperatures, it remelts at $-1.0°$(1). This water which fails to freeze at $-1.0°$C. is definite in quantity for each soil and is the same as the wilting point.

It seems that the dilatometer method should be more accurate and more reliable in the general run of soils than the direct method for the following reasons:

The dilatometer tends to give absolute results because when the soil is frozen at $-10°$C. and then placed in $-1.0°$C. only that portion of the water remains frozen on which the soil exerts a tremendous influence through its adhesive and other forces. This unfrozen water at $-1.0°$C. is a definite value for each soil and is influenced mainly by the surface forces of the soil.

The wilting point as determined by the dilatometer method is not influenced by varied climatic factors.

In stickly clays and other soils the dilatometer should give more reliable results than the direct method because with the latter it is difficult to obtain uniform moisture throughout the soil column in which the plant grows.

The validity of the dilatometer method to determine the wilting point is further supported by the results of studies of the freezing point depression, as obtained by Bouyoucos (2), Veihmeyer (4), and Schofield (3).

SUMMARY

The dilatometer method is presented as a very rapid and accurate indirect method for determining the permanent wilting point of soils.

The dilatometer method has been compared with the direct method for determining the wilting point of soils, and the results show that the two methods agree closely.

[2] In this comparative study the writer is very grateful to Dr. F. J. Veihmeyer for furnishing him many samples of soils on which he had determined the permanent wilting point by the direct method, using the plant as an indicator.

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PLATE 1

DILATOMETER USED IN DETERMINING THE WILTING POINT OF SOILS

This special dilatometer is manufactured by the American Instrument Company, Silver Springs, Maryland.

DIRECTIONS FOR MAKING MECHANICAL ANALYSES OF SOILS BY THE HYDROMETER METHOD[1]

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Since the hydrometer method is now so widely used for making mechanical analyses of soils, it has received further attention and study with the idea of possible improvement. As a result of further researches, the following improvements or changes have been made in the method:

A new type of dispersing machine (pl. 1) is now used, which is more convenient, less noisy, more durable, and more efficient than the original mixer.

A new hydrometer has been made which has a streamlined bulb, comes to equilibrium quicker, and is more accurate at the lower range of the scale than was the original hydrometer.

A new cylinder without lip is now used. This affords a better contact and consequently a better sealing between the palm of the hand and the mouth of the cylinder when the soil suspension is being shaken.

The cup has been improved in a few minor details.

Several minor changes in the technique add to the accuracy of the method.

The following is the latest procedure developed for making mechanical analyses of soils by the hydrometer method: Add 50 gm. of fine-textured soils or 100 gm. of the sand, based on oven-dry condition, to the dispersing cup. Fill the cup with distilled water to about 1½ inches of the top. Add to the contents 5 cc. of a solution of sodium silicate or water glass (2) having a hydrometer reading of 36, at 67°F., and 5 cc. of saturated and filtered sodium oxalate. If the soil is in lumps, sufficient time must be given to slake and to soak. As a matter of fact, it is well to allow all soils to soak for about 15 minutes before they are dispersed. The soils should always be air-dry, because in the wet condition they do not slake. The soaking can be done in a separate vessel and the material then washed into the cup. Connect the cup at the point where it is marked "attach here" to the stirring motor, and stir the contents for 5 minutes for sands and 10 minutes for all other soils. Those soils, however, which are recognized as difficult of dispersion should be dispersed for 20 or 30 minutes. The sands should not be stirred more than 5 minutes because they seem to undergo abrasion.

Pour and wash the contents into the special cylinder. If 50 gm. of soil is used, fill the cylinder to the lower mark with the hydrometer in it. If 100 gm. of soil is used, fill the cup to the upper mark with the hydrometer in it. Only distilled water should be used. Then take the hydrometer out, place

[1] Published by permission of the director. Station article No. 269.

the palm of one hand on the mouth of the cylinder, and shake the contents vigorously, turning the cylinder upside down and back several times. The palm when dry forms the best seal. Place the cylinder quickly on a stool or table and note the time immediately. At the desired period put the hydrometer in the suspension column, record the reading at the top of the meniscus, and then take it out again. Since there is a tendency for slight amounts of soil material to settle on the shoulder of the hydrometer, it is better not to leave it in continuously for all readings. Each time the hydrometer is used, it should be clean.

At every hydrometer reading, the temperature of the suspension should be measured. Great care must be taken, however, not to disturb too much the suspension column in putting in and taking out the hydrometer and the thermometer. For every 1°F. above or below 67°F., apply a temperature correction of 0.2 graduation on the hydrometer. This temperature correction, however, is only an approximation, as it tends to vary somewhat with extreme concentrations of soil suspensions and also with extreme variations of temperature. The most accurate hydrometer readings are taken near the temperature of 67°F., which is the temperature at which the hydrometer was calibrated in actual soil suspensions. Extreme temperatures such as 100°F. and 50°F. should be avoided. If possible, temperatures should be above 67° rather than below. For temperatures above 67°F., the corresponding amount of correction is added to the hydrometer reading, and for temperatures below 67°F., the corresponding amount is subtracted. The corrected hydrometer reading is then divided by the dry weight of soil taken and multiplied by 100, the result being the percentage of material still in suspension.

The hydrometer method cannot subdivide the sands into their various fractions but can only determine their combined or total amount. Experimental tests show that by taking a hydrometer reading at the end of 40 seconds the total combined sands of a soil can be ascertained almost to the same degree of accuracy as by the wet sieve method (sieve No. 270 with 0.053-mm. openings). A correct reading can be easily made in this short period except in some organic soils which have a tendency to produce froth at the top of the column after being shaken, in which case a correct hydrometer reading is not always possible. One way to overcome this difficulty is to add a drop or two of amyl alcohol after shaking. The procedure to be followed in determining the sands is as follows: After the soil has been shaken and the cylinder is placed on the table, a drop of amyl alcohol is quickly dropped at the top of the suspension column, one side of the cylinder wall is wiped clear so as to permit easy reading of the hydrometer, the hydrometer is placed into the suspension and steadied by hand, and at the end of 40 seconds the reading on the hydrometer is recorded.

Where a subdivision of the sands is required, the following procedure may be used: At the conclusion of the final hydrometer reading, the suspension is washed on a No. 270 sieve. That portion retained on the 270 sieve is dried

and analyzed on a set of sieves, consisting of one each of Nos. 20, 40, 60, 140, and 200.

The hydrometer was calibrated in actual soil suspensions consisting of an average loam soil. Its readings, therefore, are quite accurate.

A unique feature of the hydrometer method is the fact that the personal element is reduced to a minimum. The one important thing to watch is the condition of the paddle on the stirring rod. Practically the whole success of the hydrometer method depends upon a complete dispersion of the soil, and a complete dispersion can be obtained only when the paddle on the stirring rod or shaft is in good condition. The paddle that has been used heretofore in the method wears out very easily and becomes flat and small. In this condition its efficiency is practically gone, the soils are not properly dispersed, and consequently the results are not correct. In order to eliminate this serious weakness in the method, a new paddle or button has been adopted and placed on the stirring rod. When this paddle becomes worn it can be unscrewed from the shaft and replaced with a new one. This arrangement, besides providing a paddle that is wear-resistant, also obviates the necessity of changing the stirring rod in order to change the paddle, as has been the case heretofore. The paddles are inexpensive and can be changed frequently. It is strongly urged upon all those who are now using the old stirrer to replace it with the new one shown in plate 1.

Another important point is that the cup should have baffles in it, for without the baffles the soils can not be dispersed easily.

To calculate the conventional amount of combined sand (1.0–0.05 mm.), silt (0.05—0.005 mm.), and clay (0.005–000 mm.) and the finer clay (0.002–0.000 mm.) as determined by the hydrometer method, the procedure is as follows: The corrected hydrometer reading at the end of 40 seconds is divided by the amount of absolute dry soils taken and is multiplied by 100. The result is percentage of material still in suspension at the end of 40 seconds. This percentage is subtracted from 100, and the result is the percentage of material that settled out at the end of 40 seconds, which is supposed to represent all the sand in the soil. The corrected hydrometer reading at the end of 1 hour is also divided by the weight of soil sample and multiplied by 100. The result is percentage of material still in suspension and is considered to be the conventional clay (0.005–0.000 mm.). The percentage of the conventional silt is obtained by subtracting the clay from 100 and, from this result, subtracting again the percentage of sand. To obtain the finer clay (0.002–0.00 mm.) it is only necessary to divide the correct hydrometer reading at the end of 2 hours by the weight of the soil sample and multiply by 100.

From all studies and examinations thus far made, the hydrometer method appears to be reasonably accurate and reliable on all soils that are properly dispersed and properly stabilized by the reagent (1).

In peats and mucks, the hydrometer method is, of course, not applicable. In mineral soils containing a moderately high organic matter content, the

method is accurate provided the organic matter is well decomposed and is completely dispersed. Mineral soils containing a very high organic matter content in an incompletely decomposed state should be first treated with hydrogen peroxide.

In materials such as bentonite and fullers' earth the hydrometer method may not be very successful because these materials tend to gelatinize and emulsify and cause the hydrometer to stick and not to float normally and freely as it does in a true suspension.

It is advisable to wash very alkaline soils before using them for analysis, or a correction should be applied for the salts if present in appreciable amounts.

By taking hydrometer readings continuously or every so often, a complete distribution curve of size particles of soils and their respective amounts can be readily and reliably obtained (2).

Note: On account of the many inquiries received as to the price of the hydrometer method outfit, and where it may be obtained, the following information is given: The dispersing machine and cup (cost, about $22) are handled by the Hamilton Beach Co., Racine, Wis.; the hydrometer and cylinder (cost, about $5) are handled by the Taylor Instrument Co., Rochester, N. Y.

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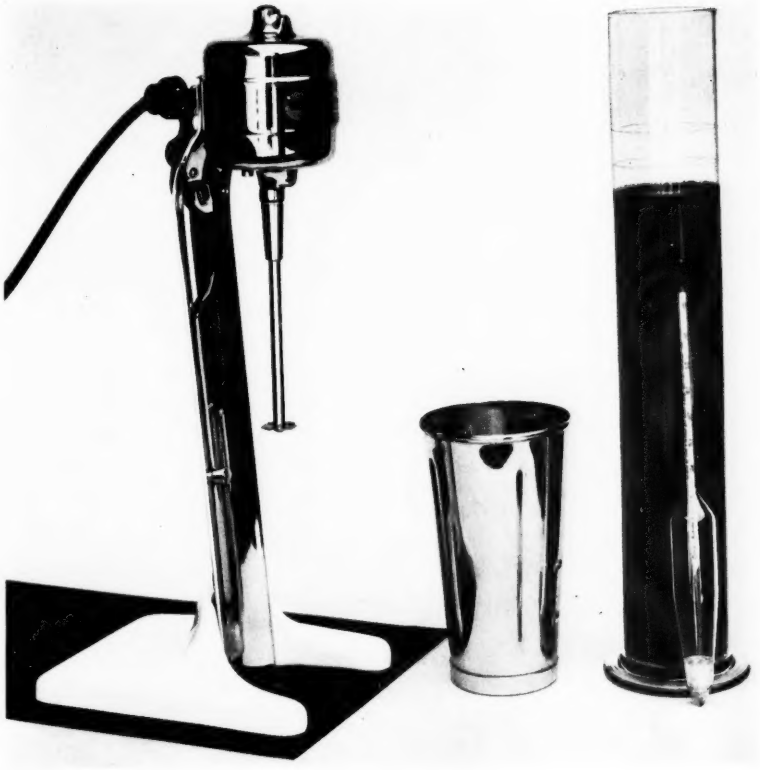
PLATE 1

Improved Dispersing Machine, Hydrometer, Cup, and Cylinder, Used in the Hydrometer Method for Making Mechanical Analyses of Soils

MAKING MECHANICAL ANALYSES OF SOILS

GEORGE JOHN BOUYOUCOS

PLATE 1



PHYSIOLOGICAL STUDIES ON RHIZOBIUM: VI. ACCESSORY FACTORS[1]

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The recent studies of Allison, Hoover, and Burk (1, 3, 9) concerning an accessory growth factor for Rhizobium have suggested a possible explanation for many difficulties encountered in the culture of these organisms. These workers reported that several species of the rhizobia were unable to make any appreciable growth in a synthetic (sugar-mineral-nitrate) medium prepared from highly purified materials. The failure to grow in such a medium was attributed to the absence of a factor essential for the respiration and growth of the organisms. They found a stimulative effect upon the rhizobia with additions of such substances as yeast extract, cane molasses, and humic acid and explained this as due to the presence of appreciable quantities of a factor which they designated as "coenzyme R."

Repetition of much of the work of Allison and Hoover, employing a medium similar to that used by those workers, has confirmed their results (12). Repeated attempts to culture various species of the rhizobia through several successive transfers in such a medium were unsuccessful if the sugar used was relatively pure.

Werkman (14) was able to culture *Rhizobium leguminosarum*, as well as several other organisms, however, through a large number of consecutive transfers in a wholly synthetic medium. He concluded that if accessory substances are needed by the organisms studied they are able to synthesize them.

The medium employed by Allison and Hoover (1) had the following composition: K_2HPO_4 — 0.7 gm., KH_2PO_4 — 0.3 gm., NaCl — 0.2 gm., $MgSO_4 \cdot 7H_2O$ — 0.2 gm., $CaSO_4 \cdot 2H_2O$ — 0.1 gm., distilled water — 1000 gm. The medium was filtered after standing 2 or more days, and 1 per cent sugar and sufficient KNO_3 to give 5 mgm. of nitrogen per 25 cc. of medium were added. For convenience this medium has been designated "medium A." The basal medium used by Werkman (14) differed in some respects from that of medium

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A. It was as follows: K_2HPO_4 — 2.0 gm., $MgSO_4 \cdot 7H_2O$ — 0.1 gm., $CaCl_2$ — 0.1 gm., $FeCl_3$ — trace, $(NH_4)_2SO_4$ — 2.0 gm., distilled water — 1000 cc. In the present investigation, this has been designated "medium B."

The reason for the different results obtained by Werkman and by Allison and Hoover was investigated by conducting a series of continuous culture experiments with the two media employed by the different workers. Sucrose c.p. was used as an energy source in both media. Experiments were conducted in 250-cc. Erlenmeyer flasks, each containg 25 cc. of medium. The media were inoculated in triplicate. The first inoculation was with a small quantity of culture from growth on yeast extract agar slants. At 4-day intervals the turbidity of cultures was observed, and transfers of 1 cc. of each culture were made into a similar medium. It was found that *Rh. meliloti*, *Rh. trifolii*, *Rh. japonicum*, *Bacillus subtilis*, and *Bacillus mycoides* were unable to continue growth through more than two or three transfers in medium A, but did continue to grow in medium B.

The composition of the two media differed principally in the nitrogen source and also in the presence of $FeCl_3$ in medium B. The addition of $FeCl_3$ to medium A greatly facilitated the growth of the organisms. The substitution of $(NH_4)_2SO_4$ for KNO_3 promoted much better growth of the organisms in the first few transfers. After that the organisms seemed to be adapted equally well to the two forms of nitrogen. In no case would they continue to grow, however, without the addition of a small quantity of available iron.

Allison and Hoover (1) found that the medium which they used contained usually enough iron as impurities to supply the needs of the rhizobia. In the present investigation several tests indicated, however, that the materials used in making the media did not contain enough iron to supply the needs of these organisms.

A review of the literature showed that ferrous and ferric iron have been very generally employed in bacterial media; but few indications were obtained as to which form was preferable for the organisms being cultured. The iron compounds most commonly used have been $FeSO_4$ and $FeCl_3$. An experiment was conducted to determine which of these compounds is most desirable for the rhizobia and also to find the optimum concentration of iron for their growth. Medium A, containing sucrose c.p. and KNO_3 (100 p.p.m. N) was employed as the basal medium. Ferric chloride and ferrous sulfate were added to the basal medium in the following concentrations of iron, in parts per million, for the different media: 0.2, 1.0, 2.0, 4.0, 20.0, 40.0, and 60.0. The basal medium, without any addition of iron, was used for the control cultures. The media were placed in 25-cc. portions in 250-cc. Erlenmyer flasks and sterilized. The organisms employed for inoculum had been freed from iron and growth factors by repeated transfers in medium A containing commercial cane sugar as an energy source. Each flask of medium received 1 cc. of inoculum containing approximately 50 million organisms. Both *Rh. trifolii* and *Rh. meliloti* were used for the tests. The cultures were incubated 4 days with intermittent shaking. At the end of that time each culture was diluted to

50 cc. with sterile 0.1 *N* HCl, and direct microscopic counts were made employing a Petroff-Hausser bacteria counting chamber. In nearly every instance the triplicate cultures agreed closely in numbers of organisms. The average number of cells developing per cubic centimeter in each medium are shown for *Rh. trifolii* in figure 1. The results obtained for *Rh. meliloti* were closely similar to those shown in figure 1 except that the optimum concentration for $FeSO_4 \cdot 7H_2O$ was at 5 p.p.m. of iron.

Figure 1 and the data obtained for *Rh. meliloti* indicate that in optimum concentrations $FeCl_3 \cdot 6H_2O$ promotes a more rapid growth of *Rh. meliloti* and *Rh. trifolii* than does $FeSO_4 \cdot 7H_2O$. The optimum concentration of iron was 10 p.p.m. in all cases except the one noted. In concentrations of iron greater than 40 p.p.m. $FeSO_4 \cdot 7H_2O$ depressed the growth of both species of the organisms; in 60 p.p.m. of iron $FeCl_3 \cdot 6H_2O$ depressed the growth. Since these results indicated that the chemicals employed in making up the media contained insufficient iron to promote optimum growth of the organisms, $FeCl_3 \cdot 6H_2O$, in a concentration of 5 p.p.m. of iron, was employed in the media used in further investigations. In greater concentration the iron precipitated some of the constituents of the medium.

POTASSIUM NITRATE AS A NITROGEN SOURCE

Although iron greatly improved the growth of the rhizobia in medium A the addition of it did not enable the organisms to maintain growth when carried through repeated transfers in such a medium with sucrose c.p. as the energy source. It has been observed that a small quantity of inoculum of the nodule bacteria often fails to grow when transferred from a yeast extract agar slant into a KNO_3-sucrose c.p. medium. Allyn and Baldwin (4, 5) have shown that KNO_3 poises bacterial media at a potential so high as to be distinctly unfavorable for the growth of the rhizobia. From the data reported by these investigators it seems that other compounds employed as sources of nitrogen might promote better growth of the nodule bacteria than KNO_3.

The growth of various species of Rhizobium in a nitrate-sucrose medium was compared with their growth in media differing only in the nitrogen source employed. Three basal media were prepared containing the mineral salts of medium A (modified by the addition of $FeCl_3 \cdot 6H_2O$ in a concentration of 5 p.p.m. of Fe) with KNO_3, NH_4Cl, or asparagin, in concentrations of 100 p.p.m. of nitrogen, as the respective nitrogen sources. Two media were then made from each basal medium by the addition of sucrose c.p. and commercial cane sugar. The media were distributed in 25-cc. portions in 250-cc. Erlenmeyer flasks. The first inoculation was made with a small quantity of culture from yeast extract-agar slants. The liquid cultures were incubated at 28°C. with frequent shaking. At 4-day intervals the turbidity of the cultures was observed, and transfers of 1 cc. of each culture were made to a flask of similar medium. The relative growth, as indicated by the turbidity of the cultures, for several consecutive transfers is shown in table 1.

The data of table 1 show that *Rh. meliloti* and *Rh. japonicum* failed to make

TABLE 1

Continuous growth of rhizobia in medium A plus different sources of nitrogen and sucrose c.p. or cane sugar

TRANSFERS AT 4-DAY INTERVALS	RH. MELILOTI 132						RH. TRIFOLII 206					
	KNO_3		Asparagin		NH_4Cl		KNO_3		Asparagin		NH_4Cl	
	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.
1	+++						++++					
2*	+++	+	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
3	++	+	++++	++++	++++	++++	++++	+++	++++	+++	++++	++++
4	++	−	++++	++++	++++	++++	++++	++	++++	++++	++++	++++
5	+	−	++++	++++	++++	++++	++−	+	++++	++++	++++	++++
6†				++++		++++				++++		++++
7†				++++		++++				++++		++++

TRANSFERS AT 4-DAY INTERVALS	RH. LEGUMINOSARUM 306						RH. JAPONICUM 403					
	KNO_3		Asparagin		NH_4Cl		KNO_3		Asparagin		NH_4Cl	
	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.
1	++++								+++			
2*	++++	++++	++++	++++	++++	++++	+	+	++++	++++	++++	++++
3	++++	++++	++++	++++	++++	++++	−	−	++++	++++	++++	++++
4	+++	++	++++	++++	++++	++++	−	−	++++	++++	++++	++++
5	++	+	++++	++++	++++	++++	−	−	++++	++++	++++	++++
6†				++++		++++				++++		++++
7†				++++		++++				++++		++++

+ Indicates relative turbidity resulting from growth.

− Indicates lack of turbidity.

* Transfer 2 made from the first transfer in cane sugar and KNO_3 except in the case of *Rh. japonicum*, which was made from asparagin, cane sugar medium.

† In transfers 6 and 7 only the cultures in media containing c.p. sucrose and asparagin and ammonium chloride as sources of nitrogen were studied.

a good growth in the nitrate media. In no case, however, was there evidence of inability to grow in the asparagin or NH_4Cl media. The results also show that the four species of the rhizobia studied were able to maintain continuous growth in media composed of highly purified materials when asparagin or NH_4Cl was employed as the nitrogen source but were unable to do so with KNO_3 as the only source of nitrogen. The relative amounts of growth for only seven transfers are shown in table 1. Similar transfers were continued, however, in the asparagin and NH_4Cl media with sucrose c.p. as the energy source. After more than two months of continuous culturing there was no appreciable decrease in the growth rates of the organisms.

INFLUENCE OF REDUCING AGENTS

If the inability of the rhizobia to make appreciable growth in the nitrate-sucrose c.p. medium was due to an unfavorable potential produced by KNO_3 it is to be expected that the addition of reducing agents to the medium would greatly facilitate growth. Continuous culture experiments showed that both cysteine and thioglycollic acid, in optimum concentrations, greatly improved the ability of medium A to support the growth of the rhizobia. *Rh. meliloti* and *Rh. trifolii* produced good growth through several consecutive transfers in medium A with sucrose c.p. as the energy source if small quantities of cysteine or thioglycollic acid were added, but were unable to do so in the absence of these reducing substances.

Several experiments were conducted with *Rh. trifolii* to investigate the influence of cysteine upon oxygen consumption. The determinations were made with Warburg manometers. The organisms were prepared for inoculum by continuously culturing in KNO_3-cane sugar medium for several transfers and washing in a sterile solution of the mineral salts of medium A, one-half concentration. The Warburg manometeric technique, as given in previous articles of this series (11, 13), was employed. The oxygen consumed by the different cultures, together with the various modifications of the medium, are given in table 2.

The data of table 2 show that cysteine increased the rate of oxygen utilization of *Rh. trifolii* in media containing either KNO_3 or NH_4Cl as the nitrogen source. Assuming the change in rate of oxygen utilization of each culture for the consecutive hourly intervals as the criteria of growth, it is also seen that cysteine brought about significant increases in the growth of the organisms. A comparison of the oxygen consumed in the KNO_3 media containing cysteine and sucrose with that in the KNO_3-brown cane sugar medium shows that cysteine did not induce as great a stimulation of the organisms as might be expected from the accessory factors proposed by Allison and Hoover. In NH_4Cl media, however, the stimulative effects of cysteine were fully as great as those of brown cane sugar.

The results of the previous experiments indicate that the inability of the rhizobia to grow in the KNO_3-sucrose c.p. medium employed may be accounted

for in two ways: (*a*) The medium was deficient in iron; (*b*) potassium nitrate, in the concentrations used, poised the medium at a potential unfavorable for

TABLE 2

Oxygen consumed (cu.mm.) by Rh. trifolii during consecutive hourly intervals in medium A plus cysteine and different sources of nitrogen

Organisms for inoculum cultured in KNO_3-cane sugar medium

KNO_3 (100 P.P.M. N)						NH_4Cl (100 P.P.M. N)					
Sucrose (1 per cent) + Cysteine (per cent)					Brown cane sugar	Sucrose (1 per cent) + Cysteine (per cent)					Brown cane sugar
Control	0.0005	0.001	0.002	0.004		Control	0.0005	0.001	0.002	0.004	
13.0	14.5	13.4	15.7	13.8	16.3	14.2	15.2	16.4	16.9	16.1	17.9
12.6	14.5	14.7	14.6	13.7	18.3	14.5	15.5	15.9	15.9	15.3	17.3
13.1	14.4	14.6	15.0	13.8	22.9	14.8	15.8	16.1	17.5	15.3	19.3
13.3	15.9	15.3	16.3	13.0	27.6	15.4	16.4	16.4	19.0	17.4	20.6
13.0	17.0	15.2	16.0	13.2	28.2	15.8	15.8	16.9	20.4	17.1	20.4
13.0	17.7	15.7	17.7	13.7	38.2	15.4	16.4	17.8	28.3	20.8	24.4
12.9	17.9	15.5	17.4	13.2	47.8	15.9	16.9	17.7	31.2	21.4	26.2
10.6	20.8	16.5	16.4	9.8	58.4	16.4	16.6	16.6	36.1	23.1	27.7
11.8	27.2	15.0	17.7	10.5	68.7	15.4	17.4	17.5	45.6	28.1	30.0
10.6	34.5	13.1	19.6	10.2	60.6	16.0	17.0	18.2	52.8	31.5	35.3
12.1	42.1	16.1	24.8	10.7	58.6	16.0	19.0	20.2	46.9	39.6	40.2
14.1	51.1	20.3	31.5	14.0	57.0	20.9	21.9	25.9	43.4	40.5	47.2
19.0	56.6	28.6	36.3	17.6	49.9	27.3	27.5	31.6	33.1	44.5	52.0
169.0	344.2	214.8	259.0	167.2	552.5	219.2	231.4	247.2	407.1	330.7	378.5

TABLE 3

Numbers of Rh. trifolii after 2 days' growth in media containing various supplementary factors

(25 cc. medium inoculated with 15,000,000 organisms)

SUGAR AND SUPPLEMENTARY FACTOR	N SOURCE 100 P.P.M.	NUMBER OF ORGANISMS PER CC. (MILLIONS)		
		1	2	Average
Sucrose c.p.	KNO_3	6.06	6.64	6.35
Sucrose c.p. + sug. ext. 100 p.p.m.	KNO_3	258.40	250.80	254.60
Sucrose c.p. + sug. ext. 200 p.p.m.	KNO_3	288.80	303.60	296.20
Cane sugar	KNO_3	52.00	54.70	53.35
Cane sugar (recrystallized)	KNO_3	8.88	9.42	9.15
Brown cane sugar	KNO_3	214.40	208.40	211.40
Sucrose c.p. + *Az. vinelandii* med. 1–8	KNO_3	291.60	286.40	289.00
Sucrose c.p.	Asparagin	24.88	24.00	24.44
Sucrose c.p. + sug. ext. 100 p.p.m.	Asparagin	449.40	452.40	451.90

respiration and growth of these organisms. The data obtained seem to give no evidence that any accessory growth factor is essential for the growth or

respiration of the root nodule bacteria. It is recognized, however, that the accessory substances reported by Allison and Hoover (1) are able to induce large increases in the growth and respiration of Rhizobium in media composed of highly purified materials. This stimulative effect has been noted in media with NH_4Cl or asparagin, as well as KNO_3 as the source of nitrogen. A representative example of the stimulative effects of some of these accessory substances upon the growth of *Rh. trifolii* is shown in table 3.

The mineral salts solution of medium A was used as the basis for the various

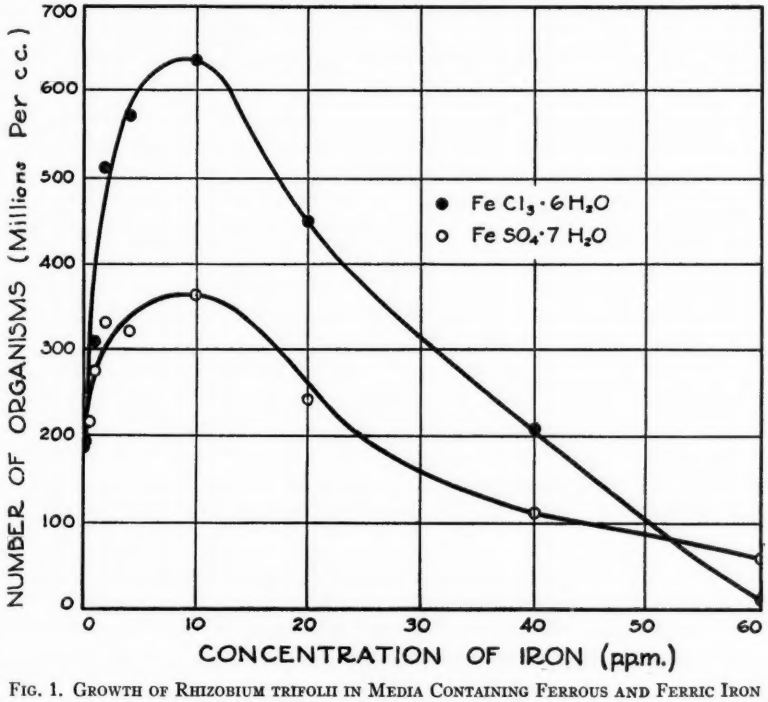

FIG. 1. GROWTH OF RHIZOBIUM TRIFOLII IN MEDIA CONTAINING FERROUS AND FERRIC IRON IN DIFFERENT CONCENTRATIONS

media shown in table 3. The organisms used for inoculum had been freed of accessory factors by culturing in nitrate-cane sugar medium. The culturing of the organisms and the direct microscopic counts were performed similarly to the procedure outlined in connection with figure 1. The sugar extract was prepared by the extraction of commercial cane sugar with absolute alcohol in the manner described by Allison and Hoover (1). A portion of the cane sugar was recrystallized from an 80 per cent solution of alcohol. The *Az. vinelandii* medium was prepared by centrifuging the organisms from a 3-week-old culture of *Az. vinelandii* growing in medium A with sucrose c.p. as

the energy source. This medium was added in a concentration of one-eighth by volume to the medium used in the test.

The data of table 3 show that the growth of the organisms was increased by the addition of the various accessory materials in just about the same degree as that reported by the previous workers. Cane sugar extract induced a 40- to 50-fold increase in the number of organisms in the KNO_3 medium and about a 20-fold increase in the asparagin medium. Studies with Warburg manometers have shown that these materials bring about 2- to 5-fold increases in the oxygen utilization of the organisms in similar media during an 8-hour period.

Some investigations have been conducted to determine the distribution and relative effects of such accessory substances. Medium A with sucrose c.p. as an energy source was used in most of the determinations. In some cases NH_4Cl or asparagin was employed as the source of nitrogen in place of KNO_3.

A water extract of alfalfa was fractionated into four parts on the basis of solubility of the parts in water-alcohol solutions of different concentrations according to the procedure of Fulmer, Duecker, and Nelson (6). The fraction soluble in 70 per cent but insoluble in 95 per cent alcohol and the fraction soluble in 95 per cent alcohol had similar stimulative effects upon the rate of oxygen utilization by *Rh. trifolii* and *Rh. meliloti*.

Water extracts of several different soils promoted an increased rate of oxygen uptake by the species of Rhizobium studied. In general the extracts of soils of high fertility were more stimulative than extracts of soils of low fertility. It was also found that heating sucrose c.p. with a solution of K_2HPO_4 or KH_2PO_4 resulted in the formation of a stimulant for the organisms.

On the basis of the preceding results it is difficult to accept the opinion that the beneficial effects of the various accessory substances studied were due to the presence of varying amounts of one specific substance. In spite of the variable nature of the stimulative agents investigated the problem has many parallels in the identification of such materials as growth factors, hormones, vitamins, and auxins for animals and plants. For this reason it is not improbable that certain groups may be found which are able to induce all of the responses in the rhizobia which have been brought about by the various accessory substances investigated.

DISCUSSION

In a recent paper, Allison and Hoover (2) reported that the addition of available iron to the medium used induced usually only a very small increase in the growth of the rhizobia. The stimulative effect of additions of ferric citrate or ferric sulfate was usually less than 10 per cent. The medium used in the work of these investigators was similar to that employed in the present investigation, except that the sugar which they used was, according to their description, a commercial sucrose containing considerable amounts of coenzyme R. Sucrose c.p. was employed in the present study. The different

materials used in the media of the two investigations make it quite probable that different quantities of iron were present as impurities. As a result, the responses to iron additions would be different. The data from this investigation indicate that although iron is essential for the growth of Rhizobium it could hardly be classed as a growth stimulant. The results of Allison and Hoover (2) do not appear to be in disagreement with this conclusion.

The use of the term "coenzyme R" has not been adopted in this paper, since no evidence has been obtained which shows that such a substance is essential for the respiration or growth of the rhizobia. Furthermore, it is felt that the term "coenzyme," as evidenced by the discussions of Hackh (7), Harden (8) and Kluyver (10), has no well-established meaning. In view of the conflicting opinions the more general terms "accessory growth substances" and "growth factors" have been employed.

SUMMARY

The addition of small quantities of iron to the nitrate-sucrose c.p. medium employed greatly increased the growth of *Rh. trifolii* and *Rh. meliloti*. Ferric chloride was found to promote greater growth of these organisms than ferrous sulfate. The optimum concentration of iron in the medium was found to be about 10 p.p.m.

Several species of Rhizobium were unable to maintain growth through repeated transfers in a mineral salts-sucrose c.p. medium with KNO_3 as a nitrogen source. If NH_4Cl or asparagin were employed as the source of nitrogen in place of KNO_3 the organisms could be continuously cultured in such a medium for indefinite periods of time.

The addition of reducing agents such as cysteine or thioglycollic acid increased the growth and oxygen utilization of the rhizobia in media composed of highly purified materials.

No evidence was found which indicates that the root nodule bacteria require any complex, unidentified substances for their growth. Many materials, however, are able to stimulate the growth and respiration of these organisms in mineral salts-sucrose c.p. media with KNO_3, NH_4Cl, or asparagin employed as sources of nitrogen.

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